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BOSTON UNIVERSITY

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Dissertation

# THE LIPOLYTIC ACTIVITY OF THE DUODENAL CONTENTS

by

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requirements for the degree of
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## INTRODUCTION

In 1910 Einhorn (60,61,62) and Gross (92), working independently, published the first practicable methods of intubating the duodenum. Hemmeter (104) had earlier published a description of a tube for reaching the duodenum. The lower end was moulded into the general shape of the stomach on the theory that such a form would favor the passage of the tip through the pylorus into the duodenum. He penned the optimistic prophesy that after the subject had swallowed it a few times he would become accustomed to it so that he would not mind it. Albeit, the tube found little use, evidently for want of subjects hardy enough to get the requisite practice. Kuhn (123) had also published a method of intubating the duodenum but his method was rigorous, not to say dangerous, and found no further use. The method of Einhorn and Gross stimulated research on the characteristics of the duodenal contents and particularly the enzymic properties. Einhorn, himself, subsequently made many contributions to the problem (63-68). Gross and his co-workers (93) published one detailed analytical procedure which largely neglected the enzymes and stressed such non-essentials as tests for albumin and bile. It met with little favor and for that reason the importance of his original contribution has not, perhaps, received the recognition it deserved. Prior to their work efforts had been made to study the enzymic activity of the duodenal contents and more particularly of the pancreas. In general they involved either stool analysis or analysis of duodenal contents obtained indirectly from the stomach as a result of regurgitation.

As early as 1890 Boas (17,18) had secured duodenal contents by draining the stomach and then forcing the duodenal material back through the pylorus into the stomach by abdominal manipulation, whence it was removed by his gastric tube. He found the material was either neutral or weakly alkaline and had a tendency to turn green on standing, a change which we now know was due to the spontaneous oxidation of the bilirubin in the bile to biliverdin (102). Boldyreff (20) had introduced a method of securing specimens from the duodenum by placing oil in the stomach and thereby inducing regurgitation. However, neither of these methods permitted a satisfactory study of the duodenal contents for obvious reasons: the specimens obtained were diluted with unknow amounts of pyloric and gastric secretions; the duodenum was not directly stimulated; the degree of regurgitation would vary widely from subject to subject. For many years an effort was made to obtain a picture of pancreatic digestion and intestinal digestion in general from the enzymic analysis of the stools.

After the introduction of the Einhorn-Gross tube permitted a direct analysis of the duodenal contents a controversy arose as to whether that method or the method of stool analysis were superior. Gaultier (81) considered the stool method distinctly superior because it gave a complete picture of digestion rather than the partial one obtained by examination of the duodenal contents alone. I should emphasize that in this consideration of stool analysis I am concerned only with enzymic activity and with no other aspects of stool examination. As late as 1934 Voegtlin, Greengard and Ivy (188) state:

> "However, we believe that it has yet to be established that any test based on duodenal drainage is of more practical value than stool analysis. This statement is made to encourage, rather than to discourage, further investigation in this field."

In general, however, the preponderance of evidence opposes that view. Deloch (52), Strauss (178), Lifschütz (129), Isaac-Krieger (113), Gubergritz (94), Gang and Klein (80), Chiray (33), and others all found the enzymic analysis of stools unsatisfactory. Gubergritz (94) felt that the proteolytic ferments in the stool stemmed largely from the leucocytes. Gang and Klein (80) decided that enzymic analysis of stools could not be expected to give any significant information in view of the many changes to which the ferments would be subjected in their passage through the intestinal tract. They also showed that bacteria produce proteolytic and lipolytic ferments. The work of Lifschütz (129) amply confirmed that latter point. In any case most workers have been unable to demonstrate lipase in the stool: Carnot and Mauban (27), Gubergritz (95), Strauss (178), Chiray, Jeandel, and Salmon (33), and others. Carnot and Bondouy (30) could not even detect lipase at the entrance to the caecum.

From the instability of lipase one would not expect ever to find it in the feces, save as it was produced by the bacteria of the colon or was liberated from an abnormally large number of leucocytes. The studies of Gyotoku (100) and Glick (83) indicate that pancreatic lipase is probably a globulin of high molecular weight. Thaysen and Norgaard (184) and Willstatter, Waldschmidt-Leitz and Memmen (200, 201) and others have shown it is very susceptible to destruction by acid. We could not, therefore, expect the lipase to survive the proteases of the intestinal canal or the acidity of

the lower part of the tract.

The foregoing discussion makes it clear that if we would determine the lipolytic activity of the duodenal contents at all we must do it on material obtained directly therefrom since indirect methods have been proven useless. The next question that arises is whether or not it is of value to analyze for lipase at all and in any case we may reasonably wonder if the question has not been satisfactorily settled in the past thirty odd years. The contention of the present work is that the analysis is of value and that the question has not been settled. I think that much of this disagreement is due to the lack of a satisfactory clinical method of lipolytic analysis and to the lack of any common denominator for comparing the results obtained by widely divergent methods. The present work is offered in the hope it may help to remedy this unfortunate state of affairs.

## METHODS OF LIPOLYTIC ANALYSIS

### Requirements

To permit a systematic critical examination of the methods of lipolytic analysis heretofore employed, I would establish the following criteria for a satisfactory clinical method:

(1) provision must be made for maintaining the optimal pH throughout the course of the reaction.

(2) the lipase must be allowed to act at its optimal temperature.

(3) inhibiting agents must be avoided and necessary activators or co-enzymes must be added.

(4) operative variables in the system must be held to a minimum and as a corollary of this, a fixed concentration of a suitable and evenly emulsified substrate must be employed.

(5) an accurate method of measuring the final extent of digestion must be available.

(6) in the interim elapsing between obtaining the specimen and the actual analysis, the enzyme must be protected from influences known to destroy it.

(7) to be clinically satisfactory the method must be simple, for otherwise it will not be used as Bondi (22) emphasized. It must consume as little time as possible and must demand apparatus available in the ordinary laboratory.

(8) the method must be designed to give as true a picture as possible of what might be expected to occur in vivo.

The validity of these criteria is readily established by an examination of the literature. We cannot, however, consider the first three without first deciding with what

lipase we are dealing in any lipolytic analysis of the duodenal contents. A brief digression is necessary to establish this point. The duodenal contents will obviously represent a mixture in different proportions of five separate secretions: salivary, gastric, hepatic, pancreatic and duodenal.

A salivary lipase active toward tributyrin has been reported by several investigators: Scheer (166), Peluffo (154), Katzenstein (118), Koebner (121), and others; but it is weak, would hardly survive the acidity of the normal stomach and in any case, would hardly be a factor in the analysis since the fasting gastric specimens were always removed at the beginning of the experiment. As a further check I employed saliva as a source of lipase and found it entirely inactive toward tributyrin under the experimental conditions of my method.

The existence of a gastric lipase is still disputed. Davidsohn (50) reported the existence of a gastric lipase and established its optimal pH as slightly on the acid side of the neutral point, namely, $2.0 \times 10^{-6}$. Willstätter and Bamann (194) and Willstätter and Memmen (197) working with extracts of the dried gastric mucosa, confirmed the existence of a gastric lipase, and discovered its peculiar property of shifting its optimal pH to the alkaline side of the neutral point as it was progressively purified by selective adsorption and elution. Meulengracht (143) with his co-workers noted the lipolytic activity of dried mucosal preparations from different sections of a hog's stomach varied. He felt the explanation might lie in the variations in the

number of lymphocytes or the extent of the surface epithelium in the regions in question. Melli and Radici (142), indeed, consider the lipase a purely cellular one which is released upon disintegration of the cells. Experiments with the dried mucosa, therefore, cannot be taken as conclusive evidence of the existence of a specific gastric lipase secreted by the mucosal cells to participate in the processes of digestion in the gastro-intestinal tract. Ando (3), Herzfeld (105), and others, however, have actually demonstrated a lipase in the gastric secretions themselves. Without attempting, then, to settle this vexing question of the existence of a gastric lipase, we may safely conclude that if a gastric lipase does exist, it is extremely weak in action in comparison with the lipase of the pancreas, and could hardly be a factor in these experiments in view of the precaution of removing the fasting gastric contents.

The existence of an intestinal lipase, elaborated by the cells of the intestinal mucosa, has been indisputably established. Slupskii and his co-workers (172) showed definite lipolytic activity in the secretions obtained from an isolated intestinal loop of man. Owles (152), using the Miller-Abbott technique of studying an isolated loop of intestine in man, clearly demonstrated the presence of a lipase in the intestinal secretions. Pierce, Nasset and Murlin (155), using dogs with isolated intestinal loops detected a lipase in the jejunum and found its action was weak. They and Lifschütz (129) give reviews of the earlier contradictory work on that problem. Baxter (10) reported that the action of the intestinal lipase was weak. These reports indicate that the intestinal lipase probably present in speci-

mens of duodenal contents does not exert much lipolytic effect in comparison with that of pancreatic lipase. This conclusion is indirectly supported by cases later to be cited, in which, as the result of pancreatic but not intestinal disease, the lipolytic activity of the duodenal contents was either absent or markedly diminished.

The only other possible source of a lipase in the duodenal contents would be the bile. Lägerlof (124) states that bile contains no enzymes. On the other hand, Freudenberg (74), working with bile obtained from the gall bladder of a sheep, found a lipase active toward tributyrin. Albeit, we cannot safely transfer that conclusion to man and, unfortunately, an analogous experiment with man could hardly be carried out for obvious reasons. Colp and Doubilet (38a) found amylase in the bile in 28.5% of their cases. They accounted for its presence in those cases on the basis of pancreatic reflux which would appear to be the most satisfactory explanation. This sheds no direct light on lipase in the bile but it does evidence the possibility of pancreatic reflux in surgical cases, a possibility strengthened by the finding of von Falkenhausen (73) that the pancreatic secretions are produced under a higher pressure than the bile and the observations of Colp and Doubilet, themselves, that post-operative oedema involves the ampulla of Vater and the biliary and pancreatic ducts and so tends to spasm of the sphincter of Odi. We thus have a satisfactory mechanism to account for pancreatic secretions in the bile draining from cholecystectomized patients. I examined three such biliary specimens, freshly obtained, and found no lipolytic activity

in two and extreme activity in the third. In combination with the evidence already cited, I think that permits disregarding any possible lipolytic activity of the bile. It is thus apparent that in determining the lipolytic activity of the duodenal contents, we are, in effect, determining the lipolytic activity of the pancreatic secretions, in the diluted form in which they occur in the duodenum.

I now return to a consideration of the criteria of a satisfactory lipolytic method of analysis. The optimal pH for pancreatic lipase has been found to cover a rather broad range. Terroine (181) reported it as slightly alkaline. Rona and Pavlovic (164) found the optimal pH with tributyrin as a substrate lay between 6.95 and 8.0. In an earlier paper Rona and Bien (163) had given the optimal pH range as from 8.3 to 9.0 but that was not with the same substrate and the nature of the substrate and of the buffer may alter the optimal pH somewhat as Dastur and Giri (49) and others have shown. Davidsohn (51) gives 8.0 as the optimum but in his work with infants he found it to be 8.5 (50). Myers and his co-workers (148) give the range as from 7.0 to 8.4. Balls, Matlack and Tucker (6) found the optimal range to be between 7.2 and 8.4. It is thus apparent that a satisfactory lipolytic method must be designed to maintain a slightly alkaline pH throughout the period of digestion.

Terroine (181) has shown the optimal temperature to be about 40° C. Balls, Matlack and Tucker (6) have shown, however, that the hydrolysis of the lower glycerides is not very dependent upon temperature. Balls and Tucker (7) have even demonstrated that pancreatic lipase will act with mea-

surable velocity in a frozen system. It would not be expected, then, that small temperature changes would markedly affect the extent of hydrolysis in a given time.

It would be useless here to consider the many inhibitors which have been found for pancreatic lipase. It suffices to consider the possible inhibitory effects of the reagents employed.in my method. The emulsifying agent was shown not to be inhibitory and the sodium bicarbonate employed to maintain the pH is normally produced by the pancreas and found in the duodenal contents (140).

The problem of activators is a much disputed one. Two of the commoner ones employed, calcium chloride and albumin, may be easily disposed of. Willstätter, Waldschmidt-Leitz, and Memmen (200, 201) employed those in the method they devised for lipolytic analysis in keeping with their principle of 'ausgleichende Aktivierung.' Höhne (108) and Ito (114) have indicated the calcium ions effect activation by precipitating the water soluble fatty acids resulting from the hydrolysis and thereby shifting the equilibrium, in accordance with the mass action principle, so the reaction proceeds to completion. In a turbidimetric method an activator of that type would not be permissible. Furthermore, in my method, the reaction will go to completion without the presence of any such activator. The opalescence contributed by albumin would likewise be unacceptable in a turbidimetric method. Its use is open to an additional objection: Bolaffi (19) has shown that peptone alone exerts a lipolytic action. That finding was confirmed by Galwialo and Simina (79). This action may explain the discovery of Falk (72) that papain

in the presence of a protein exerts a lipolytic action. Since the albumin would be added to a solution containing active trypsin, this addition would not be desirable. The question of the activation of lipase by bile is a vexing one. Sabotka (168) reviews much of the work on that problem. The consensus appears to be that bile activates lipase over and above the capacity of bile salts to act as buffers and to favor emulsification. This view is held by Lehnartz (125), Schmidt (167), Parfentjev, Devrient and Sokoloff (153), Hahn (101), Rein (160), Schmitz (171), and others. This judgment could be confirmed only by allowing lipase to act in a well-buffered system on a thoroughly emulsified substrate. McClure, Wetmore and Reynolds (136) in their method met those requirements and reported that additions of bile gave no augmentation of their results. Weinstein and Wynne (192), working under similar conditions, confirmed this report that biliary additions had no appreciable effect on the digestion. Baxter (10) reached the same conclusion even though he was working with Mellanby's (140, 141) method which calls for no buffer and uses a poorly emulsified olive oil. The method did require shaking every ten minutes during the hour's digestion which doubtless maintained a better state of emulsification than many of the other methods in which manual emulsification is employed. The reports of von Fürth and Schütz (77), Mellanby (140, 141), Chiray and his co-workers (35) and others that bile augments the action of the lipase in the duodenal contents cannot be taken at their face value since they allowed the two variables already mentioned (the buffering and surface tension effects of bile salts) to be operative at the same time they were studying a third var-

iable. Other workers with the duodenal contents have considered the addition of bile unnecessary: Lägerlof (124), Diamond and Siegel (54), Comfort (37,38) and many others. A few would add bile in cases where it is evident the biliary ducts are occluded: Berger and Schnetz (15), Lueders, Bergeim, and Rehfuss (132). Even so, Myers, Free and Beams (148) and Chiray and his co-workers (30-35) and many others have reported instances of perfectly normal lipolytic activity where there was no bile in the specimen. Mauban (135) in a study of patients with jaundice found the lipolytic activity tended to be above normal notwithstanding the absence or diminution of bile in the duodenal contents. I did not, therefore, add additional bile to my specimens for two reasons: (1) the results of others strongly indicate, but hardly prove conclusively, that it is not necessary; (2) as already noted a satisfactory clinical method of lipolytic analysis should try to give as true a picture as possible of how things might occur in vivo. If we do not test the secretions as actually obtained, we can hardly achieve that result. What does it boot the patient to tell him his duodenal contents produce fine lipolytic digestion in an Erlenmeyer flask after we have added an activator, if we must also tell him his own digestive apparatus may not appreciate the importance of producing that activator? As Leuthardt (127), McClure, Wetmore and Reynolds (136) and others have emphasized we have no way of determining enzymic concentration but can merely determine enzymic effect. Since that is the case we may as well determine the effect under conditions as similar as possible to those obtaining in the patient. Aside from the disputed role of bile

as a co-enzyme for pancreatic lipase, no evidence for a co-enzyme has been demonstrated although it has been sought by Mellanby (141), Rona and Bien (163) and others.

A very significant question that arises is whether or not tributyrin can be considered a suitable substrate for the determination of lipolytic activity. I mean here to draw a distinction between a lipase which hydrolyzes glycerides and an esterase which hydrolyzes simple esters. We can only answer this question by considering the results which tributyrin has given in comparison with other substrates. There is certainly definite evidence that steapsin is not the only ester-splitting enzyme produced by the pancreas. Kelsey (119) has reported a pancreatic cholesterase which can be readily destroyed by dilute ammonia while leaving the lipase unaffected. Falk (71) reported an esterase and a lipase in the duodenal contents distinguishable by their respective behaviour toward triacetin and ethyl butyrate. They also noted a difference in the behaviour of the two enzymes toward inhibitors. This also indicates that even simple glycerides reflect the activity of the lipase and not of the esterase. In general they obtained in any test a much greater liberation of acid from the triacetin than they did from the ethyl butyrate (from 58% to 250%). They concluded that the esterase and lipase each act toward both substrates but show a preference respectively for ethyl butyrate and triacetin. Willstätter (196, 198) and his co-workers found that pancreatic lipase would split well natural fats and tributyrin but triacetin and monoesters such as ethyl butyrate were little attacked. That result

confirmed the earlier work of Morel and Terroine (147). Willstätter and his co-workers (201) expressed the view that one and the same enzyme was responsible for splitting ethyl butyrate, olive oil and triacetin since they found that after various steps in their procedure for the purification of steapsin by adsorption their semi-purified products maintained the same relative splitting power toward those three substrates. That evidence is not strong, however, as one or more enzymes might have been destroyed in the process of purification or, assuming the presence of more than one enzyme, they might have been so similar as to react in the same way toward the reagents employed. Rona (162), using pancreatic extracts, found they would hydrolyze tributyrin very rapidly and were, in fact, the most active extracts toward that substrate. The behaviour toward monobutyrin was essentially the same which is interesting in view of Terroine's (181) finding in respect to mono-, di-, and tri-acetin derivatives: the triacetin was most readily split and the monoacetin least readily. That finding, however, could not be confirmed by Balls, Matlack and Tucker (6). Cherry and Crandall (29) do not agree with Rona and have concluded that tributyrin is not a suitable substrate for measuring lipolytic activity because it is chiefly attacked by esterases. They were not working with human duodenal contents, however, but with canine blood serum after experimentally induced pancreatic injury. It is not yet known just what the lipase which appears in the blood under those conditions is. In the light of our present knowledge of the lipolytic activity of pancreatic extracts, it is difficult to find a satisfactory interpretation of

their results. Myers, Free and Beams (148) found that human duodenal contents gave comparable results with triolein or tributyrin as the substrate. They, therefore, concluded that if the two effects were due respectively to a lipase and an esterase the two enzymes were at least present in parallel concentrations. Wolvekamp and Griffioen (203) found that after heating, the ability of their pancreatic extracts to split ethyl acetate was more completely and more quickly destroyed than was that of splitting tributyrin. Exposure to acid made that difference even more marked. They concluded from their work that there are two esterases, at least, in the pancreatic secretion, one active toward tributyrin and one toward ethyl acetate. They also suggested that there may well be more than two. Sure, Kik, and Buchanan (179) used olive oil, tributyrin, and ethyl butyrate as substrates and found the values for tributyrin and olive oil ran closely parallel although the former were generally a bit lower. The tributyrin values were much higher than those with ethyl butyrate. Bamann and Feichtner (9) reported that pancreatic lipase displayed an autocatalytic effect toward simple esters. They found no such behaviour toward tributyrin and interpreted that as due to the presence of at least two enzymes. I think the above evidence supports the conclusion that lipase attacks tributyrin readily while esterase attacks it but little, if at all. The method I used, then, employing tributyrin as a substrate, measures chiefly the action of the steapsin although some esterase activity may be involved.

The importance of adequate emulsification of the sub-

strate in any lipolytic method has been shown by several investigators: Platt and Dawson (157), Terroine (181), Anrep, Lush and Palmer (4), Vogel and Laeverenz (189), McClure, Wetmore and Reynolds (136), and others. A satisfactory method of lipolytic analysis should, therefore, eliminate the degree of emulsification as a variable which can affect the final results.

The concentration of the substrate is important and the results will vary if that be not kept constant, as was made clear by the work of Bondi (21), Leuthardt (127), and Gaultier (81).

The method of determining the final extent of digestion must be accurate and must be capable of giving reasonable agreement in duplicate determinations. The importance of that is obvious.

In working with lipase in the duodenal contents one must take care to protect it from the destructive effects of even moderate temperatures. Its relatively rapid inactivation under those conditions is probably due to the presence of active trypsin as Mellanby noted (141). Investigators have repeatedly stressed the importance of collecting the specimens in ice and analyzing them immediately: Berger (12,14), Bondi (22), Chace and Myers (28), Falk (71), Crohn (43), McClure, Wetmore and Reynolds (136), Lueders (131), Spencer (173,174), Voegtlin, Greengard and Ivy (188), and many others. Estimations of the extent of inactivation at different temperatures with time have varied. One would expect they would, if trypsin causes the inactivation, for its concentration varies and Terroine (181) has shown that the pre-

sence of bile salts accelerates the inactivation although the modus operandi in that case is a question. Bondi (21), using a titrimetric method, found that seven hours at room temperature would markedly diminish the lipolytic activity. Chiray, Milochevitch, and Lebon (35) reported that twenty-four hours on ice would not alter the results. Falk (71) confirmed that, for he found but a slight difference after sevexal days on ice. Hollander (109,110) detected a distinct loss in activity after eight hours on ice. McClure, Wetmore and Reynolds (136) obtained the same results with specimens which had stood at room temperature for twelve hours. Spencer (174) reported a 50% loss in activity after 24 hours at room temperature. Mellanby and Woolley (141) reported a loss in activity of about 10% per hour at 40° C. Terroine (181) reported that 30 minutes at 45° C. would completely destroy activity if bile salts were present, as they usually are. The low rates of inactivation reported by some of the above authors is due probably to their insensitive methods of analysis which would detect only gross changes. It would appear that the variables operative in such a heterogeneous mixture as the duodenal contents are too many to permit a quantitative determination of the degree of inactivation with time. In the light of the results reported the safest procedure would be to collect the specimens on ice and analyze them as soon after collection as possible.

The clinical significance of the seventh criterion needs no explanations. A complex method which is time-consuming and requires special and expensive apparatus will not

be used clinically, especially when the information it yields is of limited value, and the value of an enzymic analysis of the duodenal contents is definitely limited as will presently appear. It is an unattainable ideal to realize an in vitro method of enzymic analysis which will faithfully mirror in vivo activity. In vivo there are too many variables: alterations in pH, variations in the fatty substrate and the diet, in the degree of emulsification, in the intestinal motility etc. In vitro, however, we can maintain all conditions constant save one, namely, the concentration of the specimen to be analyzed. That, at least, permits a valid comparison of the strengths of different specimens under given, fixed conditions. That gives us a common denominator for comparing enzymic effects and it is only effects which we can measure. Methods which increase the variables beyond the one mentioned will merely serve to blur a none too sharply delineated picture

## Discussion of Methods in the Literature

With definite criteria for a satisfactory lipolytic method of analysis now before us we are in a position to apply them in evaluating the lipolytic methods which have already been published. I do not propose to make this discussion exhaustive but shall, in general, limit it to the leading methods and their modifications. Willstätter, Waldschmidt-Leitz, and Memmen (200,201) give a review of methods of analysis employed before they initiated their classical work with pancreatic lipase. They also give the details of their own method, based on the principle of 'ausgleichende Aktivierung' since they considered none of the previously published methods satisfactory. At about the same time they were confirmed in their judgment by von Falkenhausen (73), McClure, Wetmore and Reynolds (136), and Isaac-Krieger (113). In view of their combined judgment we may safely pay little attention to the earliest methods of lipolytic analysis.

Einhorn's first method using milk as a substrate and a piece of dyed agar as an indicator which would turn color in the presence of the fatty acid liberated in the hydrolysis, is obviously but a crude qualitative method, and was intended for no more than that. This first method (61) was later modified in that olive oil was employed as a substrate in place of milk (65) and still later (66) the oil was emulsified in agar and capillary tubes were filled with the material, which contained a dye to indicate the liberation of fatty acid by a color change in the agar medium. The activity of the lipase was expressed in the millimeters of tube length in which the dye had changed to its acid color after the ca-

pillary had been allowed to stand for a given time in the solution to be tested. The method is very rough, lacking as it does any provision for buffering and with the added variables of osmotic pressure and diffusion to effect the results.

The methods of Bondi and Salomon (23) and of Bondi and Volk (24) were improvements although no buffer was employed and the degree of emulsification was extremely variable. It was used by several subsequent investigators and produced comparable results. Bondi appreciated, as Einhorn had not, that the specimen should be protected from the destructive effect of too long exposure to room temperature. The Bondi and Volk method also avoided two sources of error peculiar to titrimetric methods and which have not always been appreciated since then. Kanitz (116) had shown that the sodium salts of the high molecular weight fatty acids hydrolyze in water to give rather strongly alkaline solutions. Phenolphthalein, therefore, would change color before all the fatty acid had been neutralized. The addition of sufficient alcohol will suppress that hydrolysis so that the titration with phenolphthalein is much more accurate. As a result of that work Bondi employed alcohol in his titrations. That also served to make the end-point somewhat sharper and easier to read. Bondi and Volk also noted that an initial neutralization of the fatty emulsion before the experiment tended to inhibit the lipase. This fact was later confirmed by McClure, Wetmore, and Reynolds (136). Notwithstanding the advantages mentioned,

Deloch (52) found this method too unreliable.

A method developed about the same time was the stalagometric method of Rona and Michaelis (161). It was devised for detecting the small amounts of lipase in the blood serum and used tributyrin as a substrate. As Willstätter, Waldschmidt-Leitz and Memmen (200, 201) indicated, the method is not applicable to the duodenal contents without a dilution of from 1:100 to 1:500. Further, as Galwialo and Simina (79) noted, the method is not so applicable with solutions which contain substances that exert a marked lowering effect on the surface tension. The presence of varying amounts of bile in the duodenal specimens would lessen the accuracy of the method and would require a time-consuming control for every experiment. In any case the method takes a good deal of time and requires special apparatus so it lacks clinical applicability, although for its original purpose it remains the most satisfactory method. Willstätter and Memmen (196) tried the method with pancreatic extracts after they had applied to it their principle of 'ausgleichende Aktivierung.' It agreed poorly with the titrimetric method which is more accurate for stronger solutions of a lipase. The original method has been variously modified without apparent advantage. Bullo and Poli (26) proposed weighing the drops but that refinement in accuracy was hardly warranted by the inherent errors of the method such as the variation in the dropping number of the control, etc. Krijksman (122) modified it by using a well-buffered solution and a home-made stalagmometer. Notwith-

standing, the method has found little use in the analysis of the duodenal contents.

The monobutyrin method employed by Isaac-Krieger (113) and others is open to the objection established by Galwialo and Simina (79) that the monobutyrin is further split during the titration.

Gaultier (81) published a method using lecithin as a substrate. It found no welcome because of the expense and variable composition of lecithin. Lueders, Bergeim, and Rehfuss (132) remedied that error in the method by employing olive oil as a substrate. They also appreciated the need for rapid analysis and that the undue exposure of the specimen to room temperature would lead to a considerable loss of activity. However, they neutralized their specimen to phenolphthalein first, which has already been shown to have an inhibiting effect upon the action of the lipase. Spencer (173) also gave a modification of the Gaultier method but he also carried out the ill-advised procedure of neutralizing to phenolphthalein first. He noted that he stopped the digestion by putting the tubes in ice-water which would hardly suffice to stop it in view of the work of Balls and Tucker (7).

Hollander (110) and Hollander and Marcus (109) devised a method representing an application of the time law of Hedin using olive oil as a substrate. It has certain objections: he initially neutralized the solution to phenolphthalein which has already been shown to be objectionable, and then added an additional measured amount of NaOH to the substrate-en-

zyme mixture and noted the time it required for the pink color to disappear. That time would vary somewhat with the buffering power of the mixture so thereby another variable is introduced. Furthermore, that added alkali would raise the pH beyond the optimal range. That same objection applies to the method of Anrep, Lush and Palmer (4) in which triacetin and a buffer was added to the solution to be tested and the time then noted for the pH to shift to a definite value as determined colorimetrically. That colorimetric comparison could hardly be very precise in the presence of a buffer.

The titrimetric method of McClure, Wetmore and Reynolds (136) seems to avoid all errors but the one inherent in the titrimetric method: in a well-buffered solution the end-point is difficult to read and without that buffering the optimal pH is not maintained throughout the course of the reaction. As Grasmann (89) noted, in the presence of bile that difficulty is further increased. McClure and his co-workers avoided that latter difficulty by diluting their specimens 1:50. That dilution gave low, final titration figures so that an error of 0.1 cc. in duplicate determinations, an error they often encountered, would amount to a rather sizable percentage error, They do not justify their boiling the specimens in the final titration, a procedure which would be inconvenient and would rapidly boil off the added alcohol. That last would tend to cause variations in the end-point by causing variations in the extent of the hydrolysis of the soaps present, as the work of Kanitz indicated (116).

The titrimetric method of Willstätter, Waldschmidt-Leitz and Memmen (201) was not designed as a clinical method of analysis and as such would be time consuming and awkward since a curve must be plotted for each determination. It was designed to show the effects of various stages in the purification of crude lipolytic extracts and as such was very satisfactory. They found it desirable in that work to add activators to be sure of 'ausgleichende Aktivierung' since they had shown that accompanying materials had a profound effect upon the lipolytic action and these were constantly being altered by the steps in the purification. Willstätter, Haurowitz and Memmen (198) reported, however, that calcium oleate and albumin had little effect on raw preparations. Vogel and Laeverenz (189) have published a very similar method, as have Weinstein and Wynne (192) although the latter modify it by adding the final aliquot to be titrated to alcohol, letting that stand, then filtering and titrating an aliquot of the filtrate. That permits a sharper end-point and so surmounts one of the inherent difficulties of the titrimetric method but it is questionable if the aliquot they titrate is a corresponding aliquot of the total fatty acid liberated in the hydrolysis since the system is not homogeneous.

Balls, Matlack and Tucker (6) devised a method which permitted adequate buffering and yet did not obscure the final end-point. It is based upon the fact originally noted by Willstätter and his co-workers (Ber. 54: 2988 (1921)), that

phenolphthalein is not sensitive to varying amounts of ammonium hydroxide in a large excess of alcohol and ether and in the presence of ammonium chloride. In a few preliminary experiments I was not able to reproduce the satisfactory results they obtained from their method, partly because duplicate titrations did not satisfactorily agree and partly because of the turbidity and intense color of the bile preparations their method calls for. The bile employed may not have been suited to the purpose as it never produced the clear solution they noted should be obtained. Furthermore, the method would never be satisfactory clinically because of the relatively large amounts of alcohol and ether it demands for the titrations. That would either make the method expensive or would require the time and special apparatus needed for a proper recovery of the alcohol and ether. For these reasons this method was not considered clinically desirable although it was felt the method met the objections which can be raised against most of the titrimetric methods.

Other little used and more unusual methods have been proposed for lipolytic analysis. Gozony, Gsell, and Hoffenreich (88) devised a turbidimetric method which depended upon the amount of casein precipitated from the solution as fatty acid was liberated in the hydrolysis of the fatty substrate. The method is interesting but of dubious practicability.

The ethyl-butyrate-glycine micro method of Glick (84) is more suited for esterase because of its substrate. It is but a titrimetric method designed to conserve materials

which is hardly necessary in working with the duodenal contents because their lipolytic action is so marked.

The molecular film method of Gorter (87) might find application with extremely weak lipolytic solutions.

Carnot and Mauban (27) devised a method involving the use of a fat emulsified in agar. This mixture was poured into Petri dishes and allowed to cool. The specimen whose lipolytic activity was to be tested was spread upon the surface and after a suitable time interval the surface of the plate was washed with copper sulphate solution. After rinsing with water, greenish blue spots could be seen where the copper had reacted with the liberated fatty acids to precipitate cupric soaps. The method was later made semi-quantitative by Mauban (134) by using the method of serial dilutions. It is an interesting method but open to objections: no buffer was employed amd could not be in such a medium; the preparation of the plates would be troublesome and there would be an inevitable arbitrary element in reading the end-point.

Gang and Klein (80) employing monobutyrin and serial dilutions read the final end-point by extracting the liberated fatty acid with an overlay of petroleum ether and benzene. That overlay, on the addition of copper acetate, would develop an emerald green color in the presence of liberated butyric acid. Leubner (126) used a turbidimetric method with triolein as a substrate. Myers, Free and Beams (148) found that tributyrin and triolein gave comparable results and tributyrin is much cheaper in a pure form. The latter has the additional advantage that temperature does not have such an effect upon its viscosity. A lowly, but eminently practical consideration

for the clinician, is that tubes which have contained a tributyrin emulsion need but be rinsed with hot water to clean them. In the use of triolein they would all have to be carefully and individually washed.

## RESULTS OF LIPOLYTIC ANALYSIS REPORTED IN LITERATURE

This review is conveniently divided into the following arbitrary sections: (1) the value of lipolytic analysis of the duodenal contents in comparison with some of the other available tests of the duodenal contents; (2) general considerations including the normal limits of variation for lipolytic activity; (3) reported results in specific conditions reviewed chronologically; (4) summarization and evaluation of results.

The only other tests of the duodenal contents with which the test for lipase may be strictly compared are those for the other two enzymes: trypsin and amylopsin. Attempts have been made to gain information regarding the enzymes indirectly by correlating their activity with the volume of secretion. They have met with little success as might have been expected in view of the dual mechanism controlling those factors. One or two generalizations have emerged but they do not have much practical value. Anrep, Lush and Palmer (4) reported that if the volume output were low, the enzymic output also tended to be low, while if the volume were high, the enzymic output also tended to be high. Berger, Hartmann, and Leubner (14) found, in contrast, that the enzymic concentration tended to be low if the volume were high, whereas if the volume were low one could expect to find almost any enzymic concentration. Gubergritz (94), Lägerlof (124), Diamond, Siegel and Gall (53), and others have reported that in general the enzymic concentration is inversely proportional to the volume. This conclu-

sion at least appears reasonable in view of the double humoral-nervous control of the gland. A Rehfuss tube obviously does not permit quantitative recovery of the duodenal contents and it is a moot point as to whether quantitative recovery could contribute information of any clinical value since in enzymic analysis we should always have to content with the varying amounts of non-pancreatic secretions present.

Probably even more fruitless have been attempts to correlate gastric secretions and the character of the duodenal contents. Einhorn (64) early emphasized the absence of any discernible correlation between the two in reporting that seven cases of achylia gastrica had enzymes present in normal concentration. Ehrmann and Lederer (59), after less extensive tests, had already reported the previous year that the pancreas was not involved in that condition. Chace and Myers (28) concluded the gastric acidity had no influence on the duodenal contents. Friedenwald and Sindler (75) and Gyotoku (98) were unable to establish any relation between the enzymes in the duodenal contents and the character of the gastric juice. Stauder (175) likewise could find no correlation between the two. Thaysen and Norgaard (184) reported higher lipolytic values than normal in cases of achylia gastrica but no significance can be attached to that conclusion because of their peculiar method of analysis. They did not remove the fasting gastric contents and their specimens were constantly being contaminated by varying amounts of acid gastric secretions being ejected through the pylorus. They ana-

lyzed their specimens without neutralizing them. Thus, in effect, they were studying the variation in lipolytic activity with pH and naturally therefore, in their cases of achylia gastrica they obtained a greater lipolytic action because in those instances they were working in the optimal pH range of pancreatic lipase. Gubergritz (95) noncommitally concluded that in achylia gastrica you may get a pancreatic hypersecretion by way of compensation or you may get a lowering of function which amounts to saying there is no correlation between the two. Goldstein (85) found in this condition that the volume of secretion was lowered but enzymatically it tended to be more active and he offered the reasonable explanation that there was less acid available to initiate the secretin mechanism. Indirect support of this view was offered by von Loeffler (130) who found that after secretin injection the pancreatic secretion was high normal or above in cases of achylia gastrica. A reasonable explanation of this is the assumption that in achylia gastrica, the pancreas, by way of compensation, learns to respond in a normal fashion to the diminished amounts of secretin which the stimulation of a less acid chyme is able to call forth. So, when a normal dose of secretin was injected a somewhat greater than normal response would result. The results of Ågren, Lägerlof, and Berglund (2), who also used secretin as a stimulus and found the response in these cases normal or above, would tend to support the above explanation. The original assumption that a correlation might be found between the status of gastric

activity and that of pancreatic activity would appear to have been based more on anatomical juxtaposition and the common digestive function of the two organs than upon any fundamental interconnection which might be expected to serve as a basis for that correlation. Certainly, to date, no such correlation has been satisfactorily demonstrated and no indirect conclusions concerning pancreatic function can be drawn from gastric analysis.

There is a lack of agreement regarding the value of tests for lipase in comparison with tests for the other two pancreatic enzymes. We cannot attach too much significance to the judgment of those who considered the test for lipase of no value in comparison with tests for the other two enzymes because the unsatisfactory nature of the available methods of lipolytic analysis may have largely accounted for their conclusions. Bondi and Salomon (23) early concluded that the determination of lipolytic activity alone offered the greatest reward for the effort involved. Grasmann (89) agreed with that view. Einhorn (66) considered the determination of tryps in the most satisfactory but as noted his method for lipase was pretty crude and inadequate. Isaac-Krieger (113) thought the determination of lipase was of value. Baxter (10) views the whole procedure of enzymic analysis as rather unsatisfactory but feels that since the enzymes are secreted in parallel concentrations the determination of only one of them will offer as much information as the method can yield. Diamond and Siegel (54) concluded from their work that the enzymes suffered first in any disturbance of the pan-

creas affecting the external secretion and that lipase and amylase were particularly apt to fall in concentration. This seems reasonable in view of the tendency of injured organs to lose first their most specialized functions. Ivy (115) concluded that lipase was more consistently low than the other two enzymes and viewed the determination of trypsin as the most satisfactory. Von Loeffler (130) reports that a decrease in amylolytic activity is the first evidence of pancreatic injury. Strauss (178), on the other hand, considered the estimation of lipase and trypsin as the most instructive. Some have reported that enzymic tests were of no value whatsoever such as D'Ignazio (55) and his co-worker. Gaither agreed with that view and as late as 1939 writes:

> "The consensus of opinion of the great majority of authors is that the estimation of pancreatic ferments in the duodenal contents and stool is, from the standpoint of diagnosis of disease of the pancreas, quite valueless. When the ferments are markedly diminished or absent the diagnosis is so self evident that laboratory tests are, as a rule, not needed."

There is a slight joker in that sweeping damnation. As will appear shortly, most workers would agree that the enzymic analysis of the duodenal contents will not diagnose pancreatic disease but in many cases it may serve to direct attention to the pancreas when its involvement was not suspected. The diagnosis will then depend on the symptoms of the patient, the history of the illness etc. since many conditions can give a depression of the ferment production.

It is generally agreed that only an absence or marked diminution in the enzymes can be viewed as significant as

Zimmer (204), Thaysen and Norgaard (184), Chace and Myers (28), Chiray, Lebon and Gozlan (31, 32, 34), Crohn (40-44), Ivy (115) and others have emphasized. Low or negative results are strengthened when several analyses yield the same findings as Isaac-Krieger (113), Comfort (37) and others have indicated. Several have stressed the view that enzymic tests can have prognostic value in following some form of pancreatic disease: Berger, Hartmann and Leubner (13,14), Berger and Schnetz (15), Lågerlof (124), and Wolodin (202). Lågerlof found repeated tests of value in following the course of a pancreatitis. Wolodin (202) would make use of them prognostically in following the course of diabetics.

The question of whether a lipolytic analysis alone of the duodenal contents will yield as much information as an enzymic analysis of that material can offer will hinge largely upon the extent to which the enzymes are secreted in parallel concentrations. Some of the earlier workers reported the enzymes were not produced in parallel concentrations: Einhorn (66), Deloch (52), Friedenwald and Sindler (75), Isaac-Krieger (113), and Katsch and von Friedreich (117). However, their methods were not accurate, as already noted, and all conclusions obtained by inaccurate methods must be held suspect. Chiray, Lebon and Gozlan (31,32,34) and Ivy (115) agree with that view but present little evidence in its support. Among the early workers Bondi and Salomon (23) were the first to support strongly the view that the enzymes were secreted in parallel concentrations. As I indicated in the discussion of

early methods, that of Bondi was probably the most satisfactory. Gyotoku (97) concluded that the enzymes may or may not run in parallel concentrations. Most of the later workers have decided that the enzymes normally are secreted in parallel concentration. Among those supporting this view are: Lågerlof (124), Leuthardt (127), Baxter (10), Ågren, Lågerlof, and Berglund (2), Anrep, Lush and Palmer (4), Diamond and Siegel (54), and Diamond, Siegel and Gale (53). The latter two groups of authors did note that various lesions could give dissociation of the enzymes and that in such instances lipase and amylase were most often involved. Anrep, Lush and Palmer (4) did not analyze for lipase but based their contention upon the parallelism displayed by trypsin and amylopsin. That point is of interest for it is the analysis for lipase which has repeatedly been condemned as unsatisfactory. The poor method of lipolytic analysis may thus have accounted in many instances for the non-parallelism encountered. Baxter (10) reviews the earlier work on dogs evidencing the parallel secretion of the three outstanding pancreatic enzymes and reaffirms that result for man. Baxter notes that since the cells of the pancreas concerned with external secretion are so remarkably uniform it is probable that one and the same cell produces all three enzymes. That suggests a reasonable basis for expecting parallelism. In the light of the evidence at present available the most reasonable view would appear to be that expressed by Diamond, Siegel and Gale (53): the enzymes are normally produced in parallel concentration but in lesions affecting the pancreas we may find dissociation.

In attempting to establish from the literature the normal limits of variation of the lipolytic activity of the duodenal contents we are faced with a well nigh insoluble problem for two reasons: (1) few methods of lipolytic analysis fulfill the criteria already set up for an accurate determination and so the results they yield are not reliable; and (2) the methods differ so much in their procedures that their results would, in any case, not be comparable. The summary in the accompanying table gives some of the results which I feel can be compared with my own.

At this point I should like to intrude a bit of reasoning which must necessarily be crude and which some may regard as dangerous but which, nonethe less, I feel has some validity. According to Harrow (102), an average daily diet may be considered to include about 80 gms. of fat. He estimates the average daily pancreatic secretion at about 600cc.. That amount of fat, if we regard it as pure tripalmitin, represents about 0.3 equivalents, bearing in mind that every molecule liberates three of fatty acid on hydrolysis. To neutralize all that fatty acid would take three liters of 0.1 N NaOH. Of the total daily pancreatic secretion it is probably reasonable to consider that only one half is effective in hydrolyzing the fat. The remainder I should view as being secreted when fat was not available for digestion or as being destroyed by the proteases before it could act or as being unable to come into contact with the poorly emulsified fat. On that crude basis, if every cubic centimeter of duodenal contents in a lipolytic test did not liberate from the fatty

substrate an amount of fatty acid requiring at least 10 cc. of 0.1 N NaOH for titration, it would be too weak in lipolytic activity to digest completely the average daily intake of fat. That neglects the undigested fat in the feces, which is not great (103) and the lipolytic effects of bacteria and intestinal lipase. The latter two cannot be very significant for failure of the pancreatic secretion alone leads to steatorrhea (182,183). We could even safely double that figure of ten cubic centimeters in view of the augmented amounts of fat in the diet which an adult can digest without leading to a significant increase in the undigested fat in the stool (103). Despite the approximations involved in that reasoning, I think it permits us to view with extreme skepticism any reported lipolytic results less than the equivalent of 10 cc. of 0.1 N NaOH per cubic centimeter of duodenal contents. I shall refer to this table again in the analysis of my own results.

The problem of the normal limits of variation in the lipolytic activity of the duodenal contents is an important one for without that information we have no clew as to what values may be considered subnormal. Several of the earlier workers reported that the lipolytic activity was remarkably constant: Bondi (21), Strauss (178), Spencer (174), Schmidt-Ott (170), and Crohn (40,43). One is forced to question the validity of their conclusions on the basis of the objections already raised to their methods. Despite this earlier work the consensus indicates that considerable normal variation is to be expected. The conclusions of many investigators support

this view: Einhorn (63-67), Zimmer (204), Leubner (126), Chace and Meyers (28), Isaac-Krieger (113), Hollander (110), Comfort ( 37), Comfort, Parker, and Osterberg ( 38), Ågren, Lågerlof, and Berglund (2), Carnot and Mauban (27), Chiray, Lebon and Gozlan (31,32), McClure, Wetmore and Reynolds (136), Voegtlin, Greengard and Ivy (188). Those who have done repeated tests on the same individual find the results are not the same: Bondi(21), Comfort (37), Einhorn (66), and Voegtlin, Greengard and Ivy (188). My own results confirm this view and one of my purposes to to establish the normal limits of variation by subjecting to statistical analysis the results obtained from the examination of a large number of normal people.

TABLE II.

| Authority | Method * | Type | cc. spec. used | Average | Results referred to cc. N/10 NaOH per cc. duodenal contents. | Time | Temp. | Substr. | Am't. |
|---|---|---|---|---|---|---|---|---|---|
| Adler | Bondi's | Titr. | 2 | 60-80 cc. | 30-40 cc. | 6-10 h. | 37° | ol. oil | 10 cc. |
| Bondi & Salomon | | " | 2 | 77.5 cc. | 39 cc. | 1 h. | r.t. | " " | 10 cc. |
| Bondi | | " | 2 | 72 cc. | 36 cc. | 7 h. | r.t. | " " | 10 cc. |
| Carnot & Mauban | | Ser. Dil. | var. | pos. with 1/16 to ½ cc. | | 1 h. | 38° | fat | ? |
| Chiray, Lebon & Milochevitch | Bondi's | Titr. | 2 | 50-60 cc. | 25-30 cc. | 1 h. | 38° | ol. oil | 10 cc. |
| McClure, Wetmore & Reynolds | | Titr. | 1/50 | .9-2.4 cc. | 45-120 cc. | 1 h. | 40° | cot. oil | 9 cc. 7% emul. |
| Strauss | Bondi's | Titr. | .5 | 20 cc. | 40 cc. | 7 h. | 38° | ol. oil | 2.5 cc. |
| Voegtlin, Greengard & Ivy | Cherry & Crandall | Titr. | 1 | 0-248 cc. (fast.)<br>49.6-260 (secr.) | 0-124 cc.<br>25-130 cc. | | | | |
| Comfort, Parker & Osterberg | " | " | 1 | 3-179 cc. N/20 NaOH | 1.5-89.5 cc. | | | | |

*their own unless so stated.

## REPORTED LIPOLYTIC ACTIVITY FOR PATHOLOGIC CONDITIONS

In the first extensive series of cases reported by Einhorn (64) there were no cases of pancreatic disease and to only four in which steapsin was absent was affixed the dubious diagnosis of 'atony of the stomach.' Even that result cannot be given too much credence in view of the crudity of his lipolytic method. That steapsin was present in normal concentration in various gastric and hepatic disorders would suggest, what we might prognosticate, namely, that pancreatic function in those conditions would be normal. Five years later Einhorn (66) reported the conclusions reached after an examination of the duodenal contents from 170 patients. He noted then that a true pancreatic achylia can exist but that it is rare and is usually the terminal stage of chronic pancreatitis. He gafe the results in the case of but one patient who was inclined to gastric distress and constipation and had a dilated stomach and enlarged liver. By implication drinking and its attendant poor diet are given as the etiological agents. The first lipolytic test on the duodenal contents of that patient showed no enzyme but it was later found after a period of rest and proper diet. The significance of the case is best summarized in his own words:

> "It is fair to assume that the disturbed pancreatic function was caused by an inflammatory process of the entire gland, involving als the islands of Langerhans, as evidenced by the presence of sugar in the urine. The rational way of living produced a change for the better, so that the inflammation subsided. The sugar then disappeared, and the pancreatic function returned to normal activity."

A general pancreatitis may thus suppress enzymic production which will once again return to normal upon subsidence of the inflammation. Three years later he reported on thirty-two additional cases, none of which, again, involved pancreatic disease. Notwithstanding, steapsin was several times absent but no safe deductions can be made from that since his method was crude and he did not protect the lipase adequately from the destructive effects of exposure to room temperature prior to the analysis.

In 1912 Hess (106) reported a study of the pancreatic ferments in infants. He refuted the old theory that marasmus was due to a lack of digestive ferments by showing they were present in even advanced cases of that disease. In a few of the more atrophic infants he detected weak lipolytic action. His contribution cannot be accorded much value since ethyl butyrate was his substrate and he was therefore determining esterase and not lipase. The following year Hess (107) reported enzymic studies in infants with acute intestinal indigestion. His results may be summarized in his own words:

> "The deficiency of lipase seemed to be to some degree characteristic of this disturbance; it is not a general characteristic of all febrile conditions, and was not met with in pneumonia or empyema. It is possible that lack of lipolytic activity in this disease should be correlated with the clinical manifestation of fat intolerance, and the metabolic studies showing a deficient absorption of fat."

That indirect confirmation of his results gives them greater credence despite his low titration figures and the relatively slight differences between his results in acute intestinal indigestion and other unrelated conditions.

In 1913 Bondi and Salomon (23) reported two cases with no lipase. They both showed 'pancreatic stools' and one also had light diabetes. He does not give the diagnoses but noted they both responded to pancreatin. They were evidently both cases of pancreatogenous steatorrhea, which is due to a diminished output of steapsin and also usually of trypsin, a point which will be referred to later. Hence his findings were undoubtedly correct. The same year Bondi (22) reported low lipolytic values for three cases of cholelithiasis and two cases of severe diabetes. Other patients with the same conditions revealed normal values.

Also in the same year Chace and Myers (28) reported results in a case with pancreatic disease. A patient with chronic pancreatitis revealed weak lipolytic action. Actually, they tested for esterase since their substrate was ethyl butyrate. Their evaluation of the test for the pancreatic ferments is worth quoting since it may still be considered essentially correct:

> "The normal variation in the activity of the pancreatic enzymes is so great, as evidenced by the data tabulated above, that only an absence of the enzymes would appear to render the juice of diagnostic value. The absence of pancreatic enzyme in the duodenal juice, would, however, be positive evidence of either pancreatitis or of non-patency of the pancreatic ducts . . ."

The ferments were normal in a case of carcinoma of the gall ducts and pylorus because the pancreatic ducts were not involved, a point confirmed at operation.

Crohn (41) reported his first results in 1913 when 27 cases were included. The woeful inadequacy of his method

which in any case measured esterase and not lipase render his results of very dubious value. In the light of later work we may accept at face value the absence of 'lipase' in three cases: two of acute pancreatitis with the diagnosis of one case confirmed at operation when the head of the pancreas was found to be markedly swollen and infiltrated; and one with a massive sarcoma involving the pancreatic ducts and confirmed at autopsy. The other cases, unfortunately, cannot be accepted at their face value. In the same year (40) he reported certain general conclusions: the ferments were low in pancreatitis; normal in gastric diseases and achylia gastrica; increased in diabetes and hepatic cirrhosis. In his cases of carcinoma of the head of the pancreas no lipase was detected. In 1915 he published three additional papers (42-44). On the basis of his studies he concluded: "Complete absence of pancreatic ferments with absence of bile, spells tumor and only tumor." He found the enzymes were usually absent in cases of pancreatic calculi although bile was present in such cases. He rightly concluded that the results in neoplasms involving the pancreas or biliary system would depend upon their location. Later work has justified his conclusions although he did not support them with detailed reports. In a second paper he reported on over 120 cases all but 17 of which he considered normal. Of those seventeen there was complete absence of ferments in six due to a neoplastic obstruction of the ducts. The other eleven revealed diminished enzymic activity due to organic or functional pancreatic disturbance. He drew the

following conclusions from his work: (1) almost all cases of pancreatitis present deficient external pancreatic secretion; (2) severe disease in a neighboring abdominal organ may cause diminution of the functional activity of the pancreas; (3) mild interlobular pancreatitis may exist without diminishing the strength of the external secretion. This is in contrast with intra-acinar inflammation, which causes severe depression of functional activity; (4) pancreatic new growths, which do not obstruct the duct, do not necessarily cause diminished secretory power. His first and last conclusions have been amply confirmed since but his evidence for the second is not convincing and the third involves an arbitrary pathological distinction of dubious value. In his third paper of 1915 (44) he covered 52 cases presenting the common symptom of jaundice. He drew the rather optimistic conclusion:

> "All in all, rules may be formulated for the occurrence of certain findings in each of the pathologic conditions usually encountered; these rules are based on the MAJORITY of the chemical examinations in such cases; exceptions to the rules, as to all biologic rules, do occur."

As will appear from later reports, the enzymic analysis of the duodenal contents cannot tell us more than whether the ducts are patent and the gland can produce the enzymes. Various conditions can lead to non-patency or to a depressed enzymic output but of the specific condition involved in a given case, the enzymic analysis can tell us nothing. In 12 cases of cholelithiasis and cholecystitis he found diminished ferments in two which he notes pointed strongly to a

complicating pancreatitis. The lipase was low or absent in seven of the twelve which is more a reflection upon his poor method of analysis than an adequate basis for profound deductions. Six of eleven cases of pancreatic malignancy revealed no ferments. He noted that in time ulcerative degeneration of the neoplasm may, in such cases, release some of the ferments. A case of acute suppurative pancreatitis revealed but traces of the three ferments. Four cases of chronic pancreatitis were diagnosed by the finding of diminished ferments in the duodenal contents. His other cases were unrelated to pancreatic disease.

In 1914 Adler (1) reported the lipolytic ferment was diminished in cases of duodenal ulcer, achylia gastrica, cholelithiasis, and in a case of carcinoma of the ductus choledochus. He does not give detailed reports or the number of cases upon which those conclusions were based. His findings in duodenal ulcer and in achylia gastrica have not been confirmed and any connection between those conditions and a disturbed enzymic production of the pancreas is not apparent. He did employ the method of Bondi which in comparison with most of the early methods of lipolytic analysis was much more satisfactory.

In the same year Gang and Klein (80) reported a case of pancreatic necrosis, confirmed at operation, which had revealed very depressed values for lipase and diastase. Another case revealing very depressed lipolytic action could be explained on the assumption of a malignancy blocking the ducts in the light of the patient's cachexia. They had no

opportunity to confirm that diagnosis, however. Their other cases did not involve the pancreas.

Grasmann in 1917 (89) reported a case of pancreatic carcinoma, confirmed at autopsy, which revealed almost no lipolytic activity.

Null (150) in 1920 made a plea for a more general use of the method of duodenal analysis and reported a case of cholelithiasis and one of cholecystitis with normal ferment values. Her findings serve to emphasize the fact that such conditions start a secondary pancreatitis or cause blockage of the pancreatic ducts only in the exceptional case. In the same year Mauban (135) reported his findings in seventeen cases of jaundice of variable origin. All but two of those cases revealed a greater action than normal.

In 1921 Isaac-Krieger (113) emphasized that duodenal analysis could not yield a sure diagnosis but that in many cases it was a definite aid. He reported 73 cases with what best might be termed assorted disorders but he did lipolytic determinations only on 27. He stressed the point that low ferment values suggest pancreatic disturbance but give no light on the nature of the disturbance. He also noted that the pancreas may be involved in disease, as by a tumor, and yet reveal a normal enzymic output.

In 1922 Deloch (52) reported finding some inconstant evidence of an involvement of the external secretion of the pancreas in cases of diabetes mellitus. He did few tests for lipase because he considered the methods too unreliable.

He noted he found evidence of a functionally established hypochylia of the pancreas in catarrhal jaundice, and an organically established hypochylia in cholecystitis, pancreatic carcinoma, and diabetes mellitus. In 1922 Gyotoku (96) reported low enzymic values in patients with typhoid fever. However, in such a disease, the test would hardly have any diagnostic value.

In the same year Katsch and von Friedreich (117) reported finding a low value for steapsin in one of five cases of diabetes mellitus. They reported low values in several other cases: achylia with anemia; nephritis; chronic diarrhea with achylia; pernicious anemia; cholecystitis; and cholecystitis with achylia. Their method had too many shortcomings to make their results of much significance.

In 1923 Lueders, Bergeim and Rehfuss (132) reported a case of diabetes mellitus which revealed no lipase on examination of the duodenal contents. The values they reported for eight cases of cholecystitis were all less than 11 cc. of 0.1 N NaOH per cubic centimeter of duodenal contents. That value, as already noted, is lower than one should reasonably expect to obtain for 1 cc. of duodenal contents and is probably due to the inadequacy of their method, because the work of later investigators has indicated that a disturbed pancreatic function in cholecystitis is the exception and not the rule. They used no stimulus to pancreatic secretion in their determinations and so even the one case of absent ferments which they reported (in diabetes mellitus) cannot

be considered too significant. Some years later in 1935 Lueders (131) reported 14 cases analyzed by his new method based on the time law of Hedin. I have already indicated that that is not an accurate lipolytic method but since its shortcomings apply alike in every determination, the results it yields with different specimens can probably be more validly compared than the results obtained by some other methods. In five cases he obtained markedly subnormal values with both specimens which he obtained after stimulation. In a sixth the one specimen analyzed revealed markedly diminished activity. The following diagnoses were given in those cases: chronic eczema with gall bladder obstruction; amebiasis and pre-diabetes; achylia and gastric tumor; achylia and an old growth with a duodenal fistula; scurvy and pre-diabetes; achylia and hyperthyroidism. The two cases with pre-diabetes are temptingly explained on the assumption a pancreatitis was present. One of those cases also had scurvy which recalls to mind Entin's (70) finding that the lipolytic activity of the liver was found closely correlatable with its vitamin C content. The two cases with a tumor and that with gall-bladder obstruction might be explained on the basis of some obstruction to the pancreatic ducts. That is, of course, hypothesis but one is obliged to seek some explanation since he did not offer one. I can devise no satisfactory explanation for the low lipolytic activity of his last case.

In 1924 Weill reported a series of cases with the following general conclusions: (1) in catarrhal jaundice the pan-

creas may or may not be involved; (2) the pancreas may be involved in biliary disease, especially cholelithiasis, in hepatic cirrhosis, and in obscure dyspeptic conditions.

In the following year Wichert and Dworjetz (193) reported the absence of lipase in a case of pancreatic carcinoma. They also reported its absence in two cases of cholecystitis, one chronic and one purulent.

In 1926 Chiray, Lebon and Gozlan (34) noted several interesting points concerning the duodenal contents. They remarked that the classical methods, at least, do not reveal any enzymic diminution in many cases of acute pancreatitis. They do not support that contention with cases, however. They suggest that positive ferment findings in cases of obstructive malignancy may be due to accessory ducts or to leakage about the neoplasm. They reported a case of pancreatic cancer in which the lipase was demonstrable. They did not, however, give its nature or location. In 1930 Chiray, Jeandel, and Salmon (33) reported a case of pancreatic cyst in which the lipase was abnormally low. They noted having detected enzymic deficiencies in several conditions including pancreatitis and cholecystitis. In 1939 Chiray and Bolgert (30) mentioned the conditions in which they have found the enzymic values low but do not support that with cases. They included cysts and pseudo-cysts, cancer, pancreatitis, and pancreatic calculi.

Thaysen (182,183) in his report of 1926 concluded that the lipolytic test of the duodenal contents was of value in distinguishing steatorrhea of pancreatogenous origin as op-

posed to other steatorrheas. He reported a series of cases of sprue but he gave the results of lipolytic tests in only two of the series. It was low in those two. Two years later Thaysen and Norgaard (184) emphasized the fact that normal variation in the lipolytic activity of the duodenal contents was so great that it was practically impossible to establish the limits between the normal and the pathological. In view of that they concluded that only the lack or almost complete lack of one or more ferments could be considered significant. Unfortunately, they did not drain the fasting gastric juice nor did they neutralize acid specimens when they were obtained. In most instances, therefore, their results are of no value. They did report one case of pancreatogenous steatorrhea in which the figure for lipase was low notwithstanding a relatively high pH.

In 1931 Hollander (110) reported 22 cases with diminished ferments. His method, representing an application of the time law of Hedin, has already been discussed and is little more than qualitative. That may have accounted for the low values which he reported in many conditions in which low values are not to be expected on the basis of the reports of others. Such are his many cases of gallstones, his cases of hepatic cirrhosis and of duodenal ulcer. He did report little or no lipolytic action in a case of pancreatitis with cholelithiasis. One other case, hepatic cirrhosis with jaundice, showed practically no lipase. If we make the assumption of the existence of a secondary pancreatitis that result

becomes reasonable. Otherwise, it is difficult to interpret his finding. The other cases all displayed a lipolytic action but one he interpreted as diminished. In view of the lack of sensitivity of his method those results must be viewed with dubiety.

In the same year Gubergritz (95) wrote that the pancreas might often be involved in cases of cholecystitis, cholangitis, and cholelithiasis. He divided diabetics into two classes: those showing a simultaneous involvment of the external secretion and those not. He noted that the former class included chiefly older diabetics in whom arteriosclerosis was the etiological agent. It is reasonable that that more general condition could easily involve both secretions. He viewed the enzymic analysis of the duodenal contents as possessed of more prognostic value than diagnostic. He noted that some of his patients who experienced diarrhea with too much fat in the diet, showed a lessened amount of lipase in their duodenal contents when their dietary fat was increased. In the same year Wolodin (202) made the same essential division of diabetics and expressed the idea the analysis of the duodenal contents would prove of definite prognostic value in following the course of such diabetics.

In 1933 Berger (11) stressed the prognostic value of duodenal analysis in following a known case of pancreatic disease but considers it of little diagnostic value especially in view of his experience of finding all kinds of pancreatic pathological changes at operation which enzymic tests had not revealed. He noted having found low enzymic curves

in five of eleven cases of pancreatic necrosis but lipase was not determined. In 1939 Berger and Schnetz (15) reported low lipolytic values in a case of metastatic involvement of the head of the pancreas. The occlusion of the ducts in that case was demonstrated at autopsy. Three cases of 'lipomatous atrophy' due to stone injuries revealed a decrease in lipase as did two cases of pancreatitis due to cholecystitis with stones. A third case similar to the latter two hardly revealed any pancreatic deficiency and at operation the pancreas was found to be normal in appearance. In other cases the findings were of little interest.

In 1935 Sure, Kik and Buchanan (179) reported a marked diminution in the activity of pancreatic lipase and esterase in uncomplicated vitamin B-1 and vitamin B complex deficiency. In 1939 Sure and his co-workers (180) concluded that only in vitamin B-1 deficiency was there a decrease in pancreatic esterase and lipase. The decrease in the former was more marked. They could establish no such digestive disturbance in other avitaminoses.

In a semi-review in 1936 Brown (25) stressed certain interesting points in connection with pancreatic cancer. He remarked that when the head is involved there is very often a disappearance of the pancreatic ferments in the duodenal contents. He believed that obstruction is the chief cause of that disappearance but remarked two other possible causes: dysfunction of the glandular tissue due to disease and the inhibitory effect upon the glandular secretion of the chronic and progressive jaundice. When the pancreatic

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ducts are not obstructed by the growth the ferment findings may be normal. He attributed that to the large margin of reserve of the pancreas which can produce a practically normal secretion even where there is considerable destruction of certain portions of its substance as in carcinoma.

In 1938 Hotz (112) pointed out the value of enzymic tests of the duodenal contents in distinguishing sprue from steatorrhea of pancreatogenous origin. In the former disease the ferments are normal while in the latter, rarer condition the lipase, especially, is diminished.

In the same year Myers, Free and Beams (148) reported low lipolytic values for seven of eight diabetics and for two other cases diagnosed respectively as: sprue, chronic pancreatitis; two cases with low values were not diagnosed. In the discussion which followed the presentation of their paper Dr. T. R. Brown noted having found an almost complete absence of ferments in several of his cases of sprue. In the light of the results he had previously reported, (25) one is tempted to question the diagnosis. Were his cases really sprue or were they cases of pancreatogenous steatorrhea? That contention is supported by Van Steenis' report in that same year of three cases of tropical sprue with normal ferment concentrations (185).

In 1939 Comfort, Parker and Osterberg (38) reported 111 cases, 17 of which were normal individuals. They found such a wide normal variation in enzymic activity that they concluded only a complete absence of enzymes could be diagnostically

significant and then only when confirmed in repeated tests. However, they felt the repeated demonstration of low values might also be significant in some cases. In their 31 cases of hepatic cirrhosis the enzymes were present in normal concentration for, as they remark, there was no reason to suspect pancreatic involvement in those patients. They found the enzymes usually absent or very low with malignancies of the head of the pancreas or of the common duct or of the ampulla of Vater which caused complete obstruction. In incomplete obstruction a low moderate concentration was found. They found the range of enzymic concentration about as wide as normal in biliary disease associated with subacute interstitial (edematous) pancreatitis; but they found a marked tendency to absence of the enzymes in chronic pancreatitis with steatorrhea. In the light of the previous discussion regarding steatorrheas, their conclusions in that respect are worth quoting:

> "In every case (6) of nontropical sprue in this series, the duodenal contents contained enzymes in apparently adequate amounts. The constant presence of enzymes in these six cases was in contrast with the impression that enzymes are often absent in nontropical sprue. The regularity of occurrence of apparently adequate amounts of enzymes inthe duodenal contents of patients with nontropical sprue also was in contrast with the regularity of their absence from the duodenal contents in cases of chronic pancreatitis with steatorrhea. Contrary to the opinions of some investigators, the examination of duodenal contents for pancreatic enzymes may be an important step in the differentiation of steatorrhea due to chronic atrophic pancreatitis and the steatorrhea of sprue, the two most important causes of steatorrhea."

In 1939 Diamond, Siegel and Gall (53) reported the results obtained in eight pathological cases. They stress the dis-

sociation of the enzymes found in their pathological cases and the consequent need for determining all three enzymes. In that connection, however, certain points are of interest. In six of their eight cases the lipase was demonstrably low. In the other two cases it was normal but in those same two cases the trypsin was normal in one and less than 25% diminished over the lowest normal value they report, in the other. The diastase is given as low in both those cases but in both of them it is well less than 20% below the lowest reported normal value. In the light of the work of others not much significance can be attached to such small deviations from the normal. In two of the six cases in which the lipase was low, it was barely below the lowest normal limit but in those two cases the tests for the other two enzymes were normal save for a low tryptic activity in one. I feel, therefore, that these results offer less convincing evidence in support of the theory of the dissociation of the enzymes in pathological conditions than they do for the contention that the determination of lipase alone can offer us about as much information as we can expect to obtain from an enzymic analysis of the duodenal contents. In their four cases with markedly diminished lipolytic activity, two were cases of steatorrhea of long standing, one was a case of gastric carcinoma with hepatic metastasis and marked cachexia and the fourth was an instance of obstruction of the pancreatic duct. In 1940 Diamond and Siegel (54) reported the results of 130 tests on 104 patients, 24 of whom were normal. In this paper they note that the enzymes appear to suffer first in any

pancreatic disturbance, particularly the lipase and amylase. They reported low lipolytic values for three patients with pancreatitis and for three with cancer of the head of the pancreas. Their uncomplicated cases of cholelithiasis gave normal figures but some of their cases with various complications revealed low enzymic concentrations, suggesting some measure of pancreatic involvement, a contention further supported in two cases by the presence of diabetes. Two cases of cancer, one involving the common duct and one the hepatic ducts gave normal lipolytic values. In three cases of pancreatic cyst the enzymic tests in one revealed marked interference with function and the other two but little. One case of splenomegaly with secondary anemia showed a diminished lipase. The cause there is not apparent unless one assumes the relative anoxemia caused a diminution of all bodily functions. An autopsied case of acute yellow atrophy of the liver (lues) revealed a marked diminution of the enzymes. In their 15 cases of steatorrhea 10 showed enzymic deficiency, especially of the lipase and 5 were normal. On that basis they would divide steatorrhea into two types: that with pancreatic insufficiency and that with no pancreatic involvement. They erroneously considered all their cases as sprue. Of four diabetics, one revealed a low lipolytic activity. Two cases with marked hepatic cirrhosis displayed a depressed enzymic activity. They concluded that in severe hepatic disease the pancreas is often involved. The reports of others, however, would suggest that that is more apt to be the exception than the rule.

In 1939 Dubois-Ferriere (58) reported a case of long standing steatorrhea in whom the lipolytic action was markedly diminished. At autopsy the pancreas was found to be large and the acini completely replaced by fat. The liver was small and cirrhotic and showed fatty infiltration.

In the same year Lägerlof wrote: (124) "The enzyme production is the first to suffer when the pancreas is damaged." He also notes that the enzymic output is depressed in the acute stage of pancreatitis but returns to normal in convalescence. He declared that a diminution in the enzymes is to be found in pancreatic stone and pancreatic cancer and in steatorrhea of pancreatic origin. He stressed the value of the enzymic test in distinguishing pancreatogenous steatorrhea from that due to other causes.

In 1939 Rauch, Litvak and Steiner (159) in reporting two cases of congenital familial steatorrhea with fibromatosis of the pancreas (one proven at autopsy) reviewed the earlier work in that disease and stressed a division of steatorrhea into idiopathic and pancreatogenous. The first class would include both types of sprue, and coeliac disease. They performed no enzymic test in their two cases but it is a safe prediction that had they done so they would have found little or no lipase.

From this review of reported cases in which a diminution of the lipolytic activity of the duodenal contents was demonstrated we can draw the following general conclusions as to the value of this enzymic test:

(1) the most clear-cut diagnostic value of an enzymic analysis of the duodenal contents would appear to lie in distinguishing pancreatogenous steatorrhea from steatorrhea of other types. This view has been supported by the work of Bondi and Salomon (23), Thaysen (182,183), Thaysen and Norgaard (184), Hotz (112), Comfort, Parker and Osterberg (38), Diamond, Siegel and Gall (53), Diamond and Siegel (54), Dubois-Ferriere (58), Lågerlof (124), and Rauch, Litvak, and Steiner (159).

(2) lipase will be found absent or in low concentration in chronic pancreatitis and in the period of exacerbation in acute pancreatitis. There are exceptions to that general principle, however, This conclusion is supported by the work of many investigators: Einhorn (66), Chace and Myers (28), Crohn (40), Gang and Klein (80), Hollander (110), Chiray, Lebon and Gozlan (31,32,34), Berger (11), Berger and Schnetz (15), Myers, Free and Beams (148), Comfort, Parker, and Osterberg (38), Diamond and Siegel (54), and Lågerlof (124).

(3) malignancies involving the pancreas directly or indirectly via metastasis will give results depending upon the location of the neoplasm. Involvement of the head of the pancreas, of the common duct or of the ampulla of Vater will tend in time to give diminished or absent lipolytic activity. This conclusion is supported by the work of Crohn (42), Adler (1), Gang and Klein (80), Deloch (52), Lueders (131), Berger (11), and Berger and Schnetz (15), Comfort, Parker, and Osterberg (38), Diamond and Siegel (54), and Lågerlof (124).

(4) pancreatic calculi and pancreatic cysts may reveal a low or absent lipolytic activity depending upon the extent of obstruction they cause and the extent to which they lead to destruction of the acinar tissue. This conclusion is indicated by the reports of: Chiray, Jeandel and Salmon (33), Diamond and Siegel (54), and Lägerlof (124).

(5) in diabetes mellitus involvement of the external secretion as revealed by low enzymic values may or may not be found. This view is supported by the findings of Wolodin (202), Gubergritz (94,95), Deloch (52), Lueders (131), Lueders, Bergeim and Rehfuss (131), Myers, Free and Beams (148), and Diamond and Siegel (54).

(6) in cholelithiasis and other involvements of the biliary tract one may or may not find evidence of a secondary involvement of the pancreas. In general that secondary involvement is probably not frequent. We find support for this in the work of Bondi (22), Adler (1), Deloch (52), Hollander (110), Gubergritz (94,95), and Diamond andSiegel (54).

(7) hepatic disease, particularly cirrhosis, is not apt to be attended by a diminished output of pancreatic enzymes although in the exceptional case it may be. Hollander (110), and Comfort, Parker and Osterberg (38) found the enzymes normal in hepatic cirrhosis. Diamond and Siegel (54) occasionally found them suppressed.

(8) evidence of a depressed enzymic output may be encountered in other conditions in which, however, the enzymic analysis

of the duodenal contents would not have much diagnostic value.

In 1921 Isaac-Krieger (113) succinctly passed judgment on the diagnostic value of enzymic analyses of the duodenal contents and his conclusions are valid today: evidence of a depressed enzymic activity of the duodenal contents bespeaks pancreatic disease but gives no clue as to the nature of the disease. The presence of a normal enzymic concentration does not preclude pancreatic disease. This analytical method is, at present, the best available to us for examining the external secretion of the pancreas.

## ANALYTICAL METHOD

When this study was initiated I considered all the published methods of lipolytic analysis and in the light of objections already raised to the common methods, chose that of Herzfeld (105) as the one which would be most convenient clinically and at the same time yield dependable results. The amounts of reagents which his method required were too small for macro determinations and they were accordingly all multiplied by a factor of five without changing their concentrations. This was done to permit making the analysis with the Evelyn colorimeter which offered the two-fold advantage of rapid analysis and objective analysis. The rapidity of analysis was a distinct advantage (I have already discussed the effect upon the results of varying the time during which an enzyme is allowed to act upon the substrate) for it was later found it took about as long to read the tubes as it did to fill them and variations in the time the enzyme was allowed to act in the different tubes was thus held to a minimum. The tributyrin emulsions prepared according to Herzfeld's directions with the five-fold increase mentioned, were unstable and even after but one hour's standing the controls failed to give constant readings when determined turbidimetrically in the colorimeter. Since I thought the emulsion might be stabilized by increasing the concentration of gum arabic which he employed as a protective colloid, several different concentrations of the gum were tried but failed to yield a stable emulsion. Even putting his emulsion

four times through a hand homogenizer, with or without the required amount of alcohol, failed to render it stable. When the logarithms of varying concentrations of this emulsion were plotted against their readings in the colorimeter, a straight line graph was obtained; but when these same concentrations of emulsion were once again read in the colorimeter after one hour's standing, the locus of the line was not the same. I concluded that Herzfeld's method would not be suitable for macro determination unless a stable tributyrin emulsion could be prepared and one which would be reproducible.

Attempts were made with four emulsifying agents in one and two percent concentration to produce a stable one percent tributyrin emulsion. They included tri-ethanol-amine stearate and three DuPont products: Modinal ES, Alkanol B, and Duponol ME. One and two grams of each detergent were respectively dissolved in two, one hundred cc., volumetric flasks approximately two-thirds filled with distilled water. One cc. of tributyrin was added to each of the resulting eight flasks, thus yielding an approximately one percent solution of tributyrin by weight, after all the flasks had been diluted to volume. The flasks were vigorously shaken and the contents were each put four times through a homogenizer. The emulsion obtained with Alkanol B* was the only one found to be stable after forty-eight hours standing. The

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*DuPont reports Alkanol B represents alkyl naphthalene sodium sulfonate.

emulsion with a final concentration of 1% Alkanol B was as stable as that with 2% so the former concentration was used.

The emulsion used for the lipolytic determinations is prepared as follows: 20.0 gms. of Alkanol B are weighed out and dissolved in a two liter volumetric flask about 2/3 filled with distilled water. The shaking must be gentle as the Alkanol B is being dissolved for otherwise an excessive amount of froth results. Twenty cc. of tributyrin are then added with a pipette and the mixture diluted to volume. The resulting coarse emulsion obtained on shaking is twice put through a homogenizer. Putting the emulsion more than twice through the homogenizer was found not to affect its stability or the turbidimetric readings which varying amounts of emulsion in a total volume of 10 cc. yielded in the colorimeter. This emulsion was found to remain perfectly stable for at least one month if kept in the ice-box. Each new lot of emulsion was checked in the colorimeter by the following procedure: six Evelyn tubes are set up and reagents added, as summarized in the table below, to yield in each instance a final volume of 10 cc.

TABLE I.

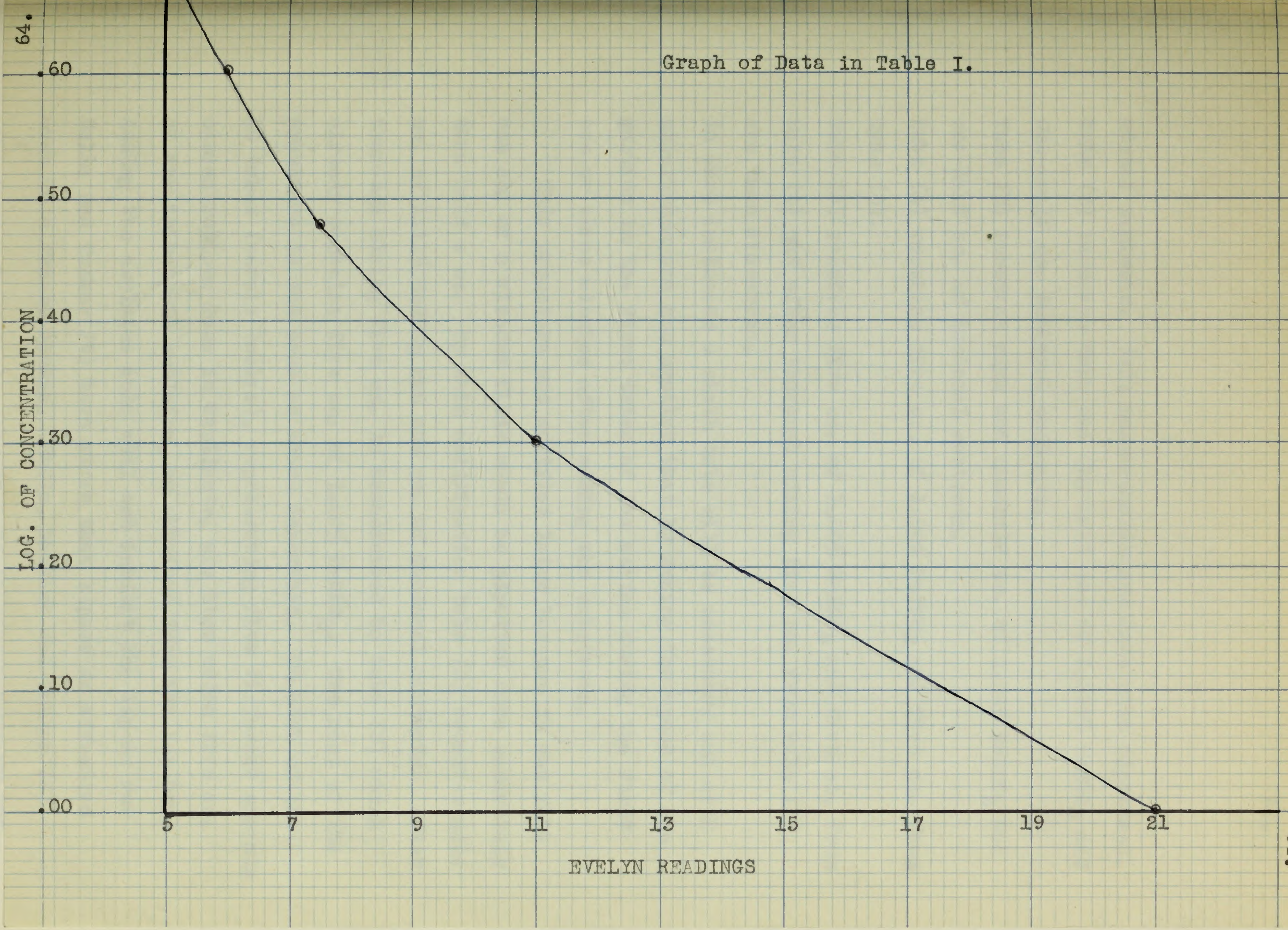
| cc. of emulsion | cc. of water | cc. of 1% $NaHCO_3$ | Typical Readings * |
|---|---|---|---|
| 0 | 5 | 5 | $100^0$ |
| 1 | 4 | 5 | $21^0$ |
| 2 | 3 | 5 | $11^0$ |
| 3 | 2 | 5 | $7^2$ |
| 4 | 1 | 5 | $6^0$ |
| 5 | 0 | 5 | $5^0$ |

*The superscripts indicate as usual quarter readings.

The first tube in the above table in all determinations served as a 'blank' and was always set at 100. The central setting was then determined and used as usual for the reference point throughout the analysis. The readings for the above standard tubes were found to be the same whether the diluent was water or sodium bicarbonate. The turbidimetric readings for different lots of emulsion prepared in the manner above described will be found to vary by not more than one quarter of a unit from the values noted in the preceding table. When the logarithms of the concentrations of emulsion are plotted against the readings obtained on the colorimeter, the resulting curve is regular and can be reproduced with different sets of standards but it is not a straight line, as may be seen from the accompanying graph. This is hardly a serious disadvantage since the graph, in any case, can be duplicated with each new lot of emulsion. It is unfortunate the first portion of the curve has such a steep slope but since most of the readings used for calculation do not fall on that portion of the curve, the error involved in interpolation is not as great as might seem at first glance. For the purposes of interpolation a large-scale graph was plotted with the ordinates representing the percentage of tributyrin digested and the abscissae the turbidimetric readings.

Each lipolytic analysis involved setting up five serial dilutions in duplicate and was done as follows:

Tube (1) receives four cc. of 1.25% $NaHCO_3$ and 1 cc. of du-



Graph of Data in Table I.

odenal contents. (All measurements are made with pipettes).
Tube (2) receives four and one-half cc. of 1.125% $NaHCO_3$ and one-half cc. duodenal contents.
Tube (3) receives nine and one-half cc. of 1.06% $NaHCO_3$ and one-half cc. of duodenal contents.
Tube (4) receives five cc. of 1.00% $NaHCO_3$ and five cc. from tube (3).
Tube (5) receives five cc. of 1.00% $NaHCO_3$ and five cc. from tube (4) and after thorough mixing five cc. from tube (5) are then discarded.
In rare instances a sixth tube will be necessary and with experience one can predict that by noting the speed of digestion in the first tubes of the series during the first two or three minutes after adding the substrate.

This procedure was modified later because it was felt one cc. of a viscous fluid could be pipetted more accurately than half a cc. By this modification tube (2) receives nine cc. of 1.11% $NaHCO_3$ and one cc. of duodenal contents. After thorough mixing five cc. is transferred to tube (3) which already contains five cc. of 1.00% $NaHCO_3$. The rest of the procedure is the same as above described. To each of the 10 tubes (rarely twelve), representing five different enzymic concentrations done in duplicate, are then added five cc. of the tributyrin emulsion, as obtained from the ice-box. Adding the emulsion cold will not inhibit the lipase for Balls and Tucker (7) have shown that lipase is effective even in a frozen system. Balls, Matlack and Tucker (6)

have shown the hydrolysis of the lower glycerides is not much dependent upon temperature. In any case the tubes will be placed in the water bath for over an hour at 40° C. and, as I shall show presently, that is the period of greatest digestion. Since time is an important factor, the emulsion is added to each pair of duplicates before being added to the next higher serial dilution. The tubes are then well-stoppered, vigorously shaken, and incubated in a water bath at 40° C. for two hours. The time interval is considered to begin the moment the emulsion is added to the first tube. A few minutes before the expiration of the two hours, the tubes are removed from the bath, wiped dry, and placed in racks beside the colorimeter, so their reading may be begun the moment the two hours is ended. The tubes should be read in the same order in which they were filled for within a minute or two it takes as long to read them in the colorimeter as to add the emulsion to them. That assures very nearly identical treatment for each tube.

Filter #565* was used for all determinations as it was felt that would help to minimize the absorption of light by varying amounts of bile present in almost all the specimens analyzed. It also helps to minimize the color contributed by the emulsifying agent which has a yellow-green tinge. An idea of the amount of bile present in each specimen was obtained by reading separately in the colorimeter a tube con-

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*According to the manufacturer this figure is the mean in millimicrons of this filter's range of maximum transmission. Ninety-five percent of the light it transmits lies in the range from 550 to 580 millimicrons.

taining one cc. of the specimen diluted to ten cc.. The effect of the color contributed by the bile would, of course, be diminished in the higher serial dilutions to an almost negligible value. The error due to varying amounts of bile was considered small since most normal specimens gave complete digestion at least as far as the second serial dilution. In clinical use one may well dispense with reading the tube just mentioned for the results indicate it yields no useful information which a note on the gross appearance and color the specimen would not give.

Digestion in the first tube of the series is normally very rapid, as adjudged by clarififcation of the tube's contents, and is usually complete within one to two minutes after the emulsion has been added. With many specimens that rapid clarification may be noted in the second and even third tubes of the series. Several investigators have shown that digestion by pancreatic lipase is very rapid during the first hour but falls off markedly thereafter. Einhorn (61), Bondi (21), Carnot and Mauban (27), Terroine (181), Willstätter, Waldschmidt-Leitz, and Memmen (200,201), Herzfeld (105), and McClure, Wetmore and Reynolds (136) have all considered an hour's digestion adequate and Rona (162) has indicated that a short period of digestion has the advantage of eliminating the danger of bacterial action. In my procedure the tubes were incubated for two hours because preliminary work indicated that the colorimetric readings of some of the tubes were shifting too rapidly at the end of the first

hour for accurate determination, a difficulty not encountered at the end of the second hour.

## SELECTION OF PANCREATIC STIMULANT

As Goldstein (85), Chiray and Bolgert (30), and Leubner (126) have emphasized, to get any true picture of the functional capacity of the digestive glands we must test their ability to respond to stimulation. The validity of this view has long been recognized in gastric analysis which may be considered an analogous determination (103). Many pancreatic stimulants have been employed in studies of the enzymic concentrations of the duodenal contents. Among them are oil, employed by Baxter (10), Demade and de Grailly (47), Bjelenky (16), and others; ether by Katsch and von Friedreich (117), and Deloch (52); various parasympathetic stimulants such as mecholyl; various foods by McClure, Wetmore and Reynolds (136) and others; and yet additional stimuli whose use and justification are too limited to consider here. The two stimuli which have most often been used are secretin and hydrochloric acid. Secretin has been employed by Ågren, Lägerlof and Berglund (2), Chiray, Lebon and Gozlan (31,32,34), Diamond and Siegel (54), von Loeffler (130) and others. Many workers have found hydrochloric acid a satisfactory stimulant: Bondi (21), Deloch (52), Dolinsky (56), Einhorn (67), Gubergritz (94), Hess (106), Leuthardt (127), Meyner (145), Vsyutochkin and Drobintzeva (186), Bjelenky (16) and others. In considering the available stimuli ether was rejected because unphysiological and because Deloch (52) had shown that it is an uncertain stimulant and can give undesirable side reactions in the patient. Oil

was rejected because of the uncertain factors involved in using as a stimulant a substrate for the enzyme for which I was testing and because Nothmann and Wendt (149) have shown that fat is normally allowed to pass the pylorus only in small driblets. That suggests that such large amounts of fat as have been employed as a stimulant are not normally present in the duodenum at any given time. Foods are unsatisfactory stimulants because of their uncertain composition and the varying degree to which they would dilute the duodenal contents. With such stimulants it would be difficult to obtain clear specimens. Mecholyl is a strong parasympathetic stimulant and in confirmation of the theory of Mellanby (140), it has been shown by Anrep, Lush and Palmer (4), Comfort (37), Diamond and Siegel (54), Ivy (115) and others that the enzymic output of the pancreas is largely under the control of the vagus. Albeit, such a stimulant is not physiological. Secretin would appear to be the stimulant of choice, analogous to the use of histamine in gastric analysis, but at the time of this work it was expensive and extremely difficult to secure. Its use, therefore, had to be foregone for practical considerations.

Hydrochloric acid, as the best available stimulant, offers distinct advantages: it is cheap and readily available in pure form; it is the normal physiological stimulant for causing the production of secretin when it comes into contact with the duodenal mucosa. Much of the work establishing that point may be found reviewed by Chiray, Lebon, and

Gozlan (31,32), by Deloch (52) and by Still (177). Some have objected to the use of hydrochloric acid as a stimulant, among them Berger (12), and McClure, Wetmore and Reynolds (136), and Chiray, Lebon and Gozlan (31,32); but the consensus would indicate that it is as good a stimulant as any when secretin is not available. I might add that the latter authors in concluding that food was more significant than the acid as a pancreatic stimulant did not exclude the effects of the hydrochloric acid which the food they administered to their patients must have called forth from the gastric glands. Accordingly, twenty cc. of N/10 HCl was employed as a stimulant in this study. It has been recognized that the mere presence of the tube in the duodenum causes some stimulation of the digestive glands. This has been reported by Berger (11,13,14), Deloch (52), Owles (152), Comfort, Parker and Osterberg (38) and others. This has been denied by Damade and de Grailly (46). Berger (13) has shown that its effect in any case does not last very long. This point must be born in mind, however, for it has been shown that the first result of stimulation of the pancreas is a washing out of pre-formed enzymes in the ducts of the gland into the duodenum, a view expressed by Ågren, Lägerlof and Berglund (2), Comfort (37), Diamond and Siegel (54), Voegtlin, Greengard and Ivy (188). The effects of stimulating the pancreas have been shown to persist for at least fifteen minutes by Chiray, Salmon and Mercier (36), Holsti (111), Voegtlin, Greengard and Ivy (188) and others. Accordingly, I made the

arbitrary choice of fifteen minutes as the time after the administration of the hydrochloric acid at which to take a specimen of the duodenal contents for analysis.

A pertinent consideration is whether or not we should gain more information by the analyses of many samples obtained at different time intervals after stimulation of the pancreas. Such a procedure has been strongly advocated by several workers. Among them are Berger, Hartmann and Leubner (13,14), Ågren, Lägerlof and Berglund (2), Chiray, Lebon and Gozlan (31,32,34), Comfort (37), Diamond, Siegel and Gall (53), Friedenwald and Sindler (75), Gubergritz (94,95) and Lueders (131). Such a procedure is bound to yield us more information concerning some aspects of pancreatic secretion such as volume and the length of time the gland is able to show an augmented output after stimulation. However, it has not yet been demonstrated that this procedure can offer any more significant information in testing for one enzyme. To make the fractional method of analysis really significant we should have to carry out the extensive procedure necessary to recover the secretions quantitatively. Even then we should be working with specimens made up of four different secretions and would have no way of knowing what fractions each contributed of the total specimen. For the present we may say that the diagnostic applicability of the fractional method of enzymic analysis of the duodenal contents remains to be shown. Of course, as already noted, absent or diminished lipolytic activity can be viewed as significant only when demonstrated in repeated tests.

## METHOD OF COLLECTING SPECIMEN

The method of intubating the duodenum has become too well known to warrent its discussion here. Excellent descriptions may be had by consulting appropriate texts or by referring to such original papers as those by Adler (1), Berger (28), Owles (152), and Bondi (22). In all cases in this series the fasting gastric specimens were removed before the tube was allowed to pass through the pylorus into the duodenum. The need for that procedure has been recognized by a number of investigators: Friedenwald and Sindler (75), Chace and Myers (28), Comfort (37), Deloch (52), and Diamond and Siegel (54) and others. With that precaution practically all specimens obtained were found to be neutral or alkaline to litmus. Spencer (174) reported that some specimens may be sufficiently alkaline to react alkaline to phenolphthalein but Einhorn reported (67) never having found such a specimen in his long series of cases. Those who have reported the specimens as acid usually did not take the precaution to remove the gastric juice - Bondi (22), Gross ( 92), Crohn (41), Thaysen and Norgaard (184) and others. The latter authors themselves noted that the acidity was due to varying amounts of acid gastric secretion being ejected into the duodenum from the stomach. In general, however, the usual experience may be summarized by a quotation from Voegtlin, Greengard and Ivy (188):

> "Immediately upon passing the pylorus, the tube began to deliver an alkaline fluid (litmus paper test used), which was transparent and nearly always tinged with

> bile. In only two instances out of the series of 22 human subjects was this control drainage entirely free of bile. In nearly every case during the collection of control samples the drainage became turbid and acid in reaction for short invervals. We interpret this reaction as being due to an opening of the pylorus and the admission of stomach contents into the duodenum, Upon alkalinization of these samples they rapidly became clear and resembled the other control samples. They were rejected for enzyme analysis, however, because of the unknown factor of dilution by gastric contents which had been introduced. It is probable that if the stomach had been constantly drained during the experiment this reaction would not have occurred."

Others have rejected for analysis any acid specimens not only because of the dilution factor but because of the susceptibility of lipase to destruction by acid - Hess (106,107), and Adler (1). The work of Waldschmidt-Leitz and Schäffner (190) made abundantly clear the destructive effect of acid. In my own series acid specimens were rejected and collection continued until a clear, neutral or alkaline specimen (litmus) was obtained. That problem, however, was rarely encountered. The gross appearance of the fluid obtained from the duodenum has been well described by many workers in the field and with almost complete agreement. Boas (17) early noted the secretion was neutral or weakly alkaline. Einhorn (60-62) confirmed that and reported only isolated cases in which the secretion was acid. He summarized his experience as follows:

> "If the capsule is in the duodenum a clear, golden yellow or watery liquid of alkaline reaction and somewhat viscid consistancy is generally obtained."

He remarked an occasional case in which the fluid was not bile-stained, an experience confirmed by Friedman (76), Voegtlin, Greengard and Ivy (188) et al. Such a specimen was occasionally encountered in this series. This general

picture of the duodenal specimen has been confirmed by Bondi (21,22), Friedenwald and Sindler (75), Zimmer (204) and others.

## EVALUATION OF METHOD

It is pertinent to consider briefly the extent to which the method of this paper satisfactorily meets the criteria earlier set up for an accurate and adequate clinical method of lipolytic analysis. The $NaHCO_3$ is present in every tube to the amount of about .0006 equivalents. If the tributyrin in every tube is completely hydrolyzed that will release about .0005 equivalents of acid. There is thus more than enough bicarbonate present to neutralize completely the acid liberated in the hydrolysis and still maintain a slightly alkaline pH. The optimal temperature for the reaction of pancreatic lipase is maintained during the experiment. The emulsifying agent used was shown not to be inhibitory to lipolytic action and the bicarbonate employed as a buffer is normally present in the pancreatic secretions(102,103). The only variable in the method is the concentration of enzyme for all other factors were kept constant. Tributyrin has been shown to be a suitable substrate for lipase. The evenness of emulsification is evidenced by the regularity with which given dilutions of different lots of emulsion yielded the same colorimetric readings. As already noted, the inherent error in analysis with the Evelyn colorimeter is not significant in comparison with other errors in any enzymic analytical method. All specimens were iced immediately upon collection and promptly analyzed, thereby reducing to a minimum any digestion of the lipase by the active trypsin present. The method is simple and requires little

apparatus. It is doubtless clinically satisfactory to omit readings in the colorimeter and to base conclusions upon the number of tubes showing complete digestion in each series. Greater accuracy than that is not necessary since it has already been made clear that only considerable variation from the normal may be viewed as diagnostically significant. With practice an analysis may be done in less than forty minutes.

## METHOD OF RECORDING RESULTS

It is apparent that mathematical conclusions regarding the enzymic strength of any specimen tested by my method can only be drawn from the first tube in the serial dilutions which does not exhibit complete digestion. From any tube which exhibits complete digestion we get the information that there was sufficient enzyme present to achieve that effect but we learn nothing of the possible excess of enzyme which may have been present over and above that needed to produce complete digestion in the two hour period of the experiment. By taking the turbidimetric readings of the first tube in each series which showed incomplete digestion and interpolating on the graph already mentioned I have obtained the concentration of undigested tributyrin. That permits calculation of the reaction velocity by substituting the values so obtained in the equation for a first order reaction:

$$K = \frac{2.303}{t} \cdot \log \frac{a}{a - x}$$

where K is the velocity constant; a is the initial concentration; and (a - x) is the concentration remaining after time t expressed in minutes. To make these velocity constants comparable they are all referred to one cc. of duodenal contents as a unit by an arbitrary assumption which appeared to be the only satisfactory method of dealing with a discontinuous curve. It was assumed that the final concentration of tributyrin in every tube showing 'complete' digestion was 10%. On that assumption the corresponding velocity constant was calculated and found to be 0.01919. In every calculation

then, this value was multiplied by the reciprocal of the dilution of the specimen in the last tube of the series showing complete digestion and to that figure was added the actually calculated velocity constant obtained in the manner noted above. The resulting figures give us a valid basis for comparing the enzyme effect of different specimens of duodenal contents. They do not give us, and are not intended to give us any basis for comparing the relative enzymic concentrations of different specimens. In any case, that latter point can be a matter only of academic interest since we have no methods of determining enzymic concentrations.

## REPORT OF RESULTS

The results of the present investigation are given in Table II. The results of fifty intubations are reported and of that fifty, seven represent repeated intubations on the same subject. For comparative purposes those results are separately given in table III. The analytical results are given for a total of fifty fasting specimens and for a total of 64 specimens obtained at various intervals after stimulation of the duodenum with 0.1 N HCl. The healthy normal subjects included fourteen young women, mostly nurses and nineteen young men, all medical students. The remaining ten subjects were all patients from the Gastro-Intestinal Clinic of the Mass. Memorial Hospital. These cases are included because in none of them was pancreatic disease suspected and they all revealed lipolytic values lying within the normal range.

With five of the subjects specimens were taken at 5, 10 and 20 minute intervals after the stimulation with dilute hydrochloric acid. In each of those five cases all three specimens revealed complete digestion in the same number of tubes. In ten other cases two specimens were taken after stimulation at 10 and 20 minute intervals (usually). In six of those 10 the two specimens gave complete digestion in the same number of tubes while inthe other four they differed by only one tube in the number in which they gave complete digestion. I therefore concluded that for clinical purposes the information obtained by testing more than one specimen

after stimulation did not warrant the trouble involved. This conclusion but confirms the views of many other workers, a point which has already been discussed.

In regard to the accuracy and sensitivity of this method two points are of interest. In the entire series of 114 analyses there were 9 cases in which the duplicate determinations did not show complete digestion through the same number of tubes. In seven of these one determination showed complete digestion through the nth tube while its duplicate determination showed well over 80% digestion in the nth tube but it was not quite complete. Because of this relatively slight difference these cases are reported in the table as revealing complete digestion through the nth tube. The other two cases in the nine are also so reported although in those instances one determination revealed somewhat less than 80% digestion but well over 60%. The specimens in those two cases were exceptionally viscous and the relatively poor agreement in their analyses is undoubtedly due to the impossibility of accurately pipetting such viscous samples. In the remaining 105 analyses duplicate determinations showed complete digestion through the same number of tubes. In interpreting from the colorimetric readings the degree of accuracy in the duplicate determinations in the first tube in each series showing incomplete digestion one must keep in mind the nature of the curve obtained on plotting the percentage of undigested substrate against the colorimetric readings. The slope is extremely steep from 100% to 80% substrate. It is much less steep from 40% to 20% and from 20%

to 0% is very moderate. Thus in the last portion of the curve a difference while in the first portion of the curve a difference of ½ a unit would amount to a percentage difference of 10%. The greatest error (case 37-10) is less than 40%. I consider this agreement satisfactory especially in view of the inherent sensitivity of the method of serial dilutions.

To aid in interpreting the significance of the normal range established in this series for the lipolytic activity of the duodenal contents the data were subjected to statistical analysis employing the formulae and tables given in Pearl. For this purpose I could use for each specimen only the number of the last tube revealing complete digestion. To subject the calculated velocity constants to a similar analysis would involve special statistical methods and would hardly yield any more significant information clinically (Prof. William Brooks--private communication). Calculations for the fasting specimens are not included because the occurrence of three such specimens revealing no action made their dispersion so great that on statistical analysis they yielded results whose interpretation could have no clinical significance. The difference between the means of the fasting and stimulated specimens in each group is not great, but the difference in degree of dispersion was considerable. The responses after stimulation were more consistent and fell in a narrower range so, I feel the statistical analysis of those data yields information of definite clinical significance.

In the latter group the limits were from 2 to 5 tubes. In the fasting specimens the limits were from 0 to 5 tubes.

TABLE IV

| No. of cases | Group | Fast. or Stim. | Mean | S.D. | P.E. | Chance of finding normal case beyond the noted limits of variation. (a) below | (b) above No. in 10,000 |
|---|---|---|---|---|---|---|---|
| 50 | Entire | F | 3.16 | | | | |
| 64 | Series | S | 3.34 | .82 | .07 | 22 | 6 |
| 40 | Healthy | F | 3.30 | | | | |
| 53 | studs. only | S | 3.80 | .72 | .07 | 1 | 11 |
| 24 | Male | F | 3.04 | | | | |
| 35 | studs. | S | 3.86 | .61 | .07 | | |
| 16 | Female | F | 3.69 | | | | |
| 18 | studs. | S | 3.67 | .88 | .14 | | |
| 10 | Patients | F | 2.6 | Too few cases to yield significant results. | | | |
| | | S | 2.4 | | | | |

*Calculated on the basis of cases revealing complete digestion only through the first or through the sixth tubes respectively. The chance of finding normal cases revealing no digestion or digestion through the seventh tube would be infinitesimally small.

It is apparent from the above table that any specimen of duodenal contents which reveals complete digestion only through the first tube when analyzed by my method is almost certainly abnormal. If it reveals no digestion we may safely say it is certainly abnormal. My method of analysis has the advantage, then, of establishing sharply a lower normal limit of lipolytic activity. Other methods have lacked this degree of sensitivity and have lead previous workers to the conclusion, as already discussed, that only an absence, or almost

complete absence, of lipolytic activity could be considered significant. With my method a specimen may reveal definite lipolytic action and yet we may say with a high degree of certainty that it is so little as to be abnormal.

The differences between the male and female students were considered but they were found to be too small to have significance in the light of the limited number of cases. Such small differences, according to Pearl (156) could be viewed as significant only with a minimal number of approximately three hundred cases.

No conclusions can be drawn from the ten hospital patients examined because the number ofcases is too few. It is interesting that the results in these cases, while all normal, fell in the lower range of normal. With a large series of cases a correlation with age might be possible for as a group they were much older than the medical students and nurses.

The relatively small differences between the means of fasting specimens and specimens obtained after stimulation are probably to be explained by the stimulating effect of the tube itself, a point I have previously discussed.

The results obtained by my method are not strictly comparable to those obtained by a titrimetric method but by interpolation figures can be obtained for comparison with those in table II. Approximately 0.0005 equivalents of tributyrin, considered acidimetrically are added to each tube in my determination. If that were completely hydrolyzed the liberated butyric acid would require five cc. of 0.1 N NaOH for neu-

## Table III a.

| Subject | Case No. in Table III | Intubation No. | Last tube showing complete digestion |
|---|---|---|---|
| 1 | 1-f | 1 | 5 |
| | 1-15 | 1 | 4 |
| | 22-f | 2 | 3 |
| | 22-15 | 2 | 2 |
| 2 | 12-f | 2 | 4 |
| | 12-15 | 2 | 3 |
| | 27-f | 1 | 2 |
| | 27-15 | 1 | not done |
| 3 | 18-f | 1 | 4 |
| | 18-15 | 1 | 4 |
| | 42-f | 2 | not done |
| | 42-5 | 2 | 3 |
| | 42-10 | 2 | 3 |
| | 42-20 | 2 | 3 |
| 4 | 21-f | 1 | 2 |
| | 21-15 | 1 | 4 |
| | 26-f | 2 | 5 |
| | 26-15 | 2 | 4 |
| 5 | 23-f | 1 | 4 |
| | 23-15 | 1 | 4 |
| | 50-f | 2 | 5 |
| | 50-10 | 2 | 5 |
| | 50-20 | 2 | 5 |
| 6 | 24-f | 1 | 5 |
| | 24-15 | 1 | 4 |
| | 34-f | 2 | 4 |
| | 34-10 | 2 | 5 |
| | 34-20 | 2 | 4 |
| 7 | 25-f | 1 | 4 |
| | 25-30 | 1 | 4 |
| | 40-f | 2 | 4 |
| | 40-15 | 2 | 4 |
| | 40-25 | 2 | 4 |

tralization. If we multiply that figure by the the reciprocal of the dilution of the specimen in the last tube showing complete digestion we obtain a figure which may be compared with those in table II. On that basis a specimen which gave complete digestion only through the first tube and partial in the second would require at least five cc. of 0.1 N NaOH to neutralize the butyric acid which one cc. of the specimen would liberate from tributyrin as a substrate. The corresponding figures for complete digestion through tubes 2, 3, 4, and 5 respectively would be 10 cc., 20 cc., 40 cc., and 80 cc. of 0.1 N NaOH. In each case, of course, the true interpolated value would lie between the one designated and the next higher since all specimens gave partial digestion in the tube following the last one in which it had given complete digestion. The results so obtained are at least of the same order of magnitude as those given in table II.

It is apparent from table III that there is a tendency for specimens obtained from the same individual at different times to reveal comparable enzymic activity. A similar tendency has been noted by Ågren, Lägerlof and Berglund (2), Chiray, Salmon and Jeandel (33). Others, on the other hand, have reported considerable fluctuation in repeated tests on the same individual: Einhorn (66), Einhorn and Rosenbloom (63), Goldstein (85), Chace and Myers (28), Crohn (43), and Comfort, Parker and Osterberg (38). However, the marked variation in the methods employed and their degree of accuracy and sensitivity make it difficult to draw any final con-

clusions. The question must be regarded as unsettled until further work has been done.

## TABLE III.

| Case No.- Fast. or min. after stim. | Last tube that shows compl. digest. | Evelyn tubes in duplicate for first incompletely digested tube. | | Aver. | % substrate undigest. | K=vel. const. calc. from 1st tube incompl. digested. | Final K |
|---|---|---|---|---|---|---|---|
| 1-f | 5 | * | | | | | |
| 1-15 | 4 | $7^{0}$ | $7^{0}$ | $7^{0}$ | 66 | .00344 | .15696 |
| 2-f | 3 | $16^{3}$ | $32^{1}$ | $24^{2}$ | 19 | .01384 | .09060 |
| 2-15 | 3 | $41^{0}$ | $46^{2}$ | $43^{3}$ | 14 | .01640 | .09316 |
| 3-f | 3 | $33^{2}$ | $33^{0}$ | $32^{3}$ | 17 | .01476 | .09152 |
| 3-15 | ** | | | | | | |
| 4-f | 4 | $5^{1}$ | $5^{2}$ | $5^{1.5}$ | 93 | .00056 | .15408 |
| 4-15 | 4 | $9^{0}$ | $8^{3}$ | $8^{3.5}$ | 49 | .00595 | .15947 |
| 5-f | 4 | $6^{3}$ | $6^{2}$ | $6^{2.5}$ | 72 | .00275 | .15627 |
| 5-15 | 4 | $7^{0}$ | $7^{0}$ | $7^{0}$ | 66 | .00344 | .15696 |
| 6-f | 3 | $18^{0}$ | $16^{1}$ | $17^{.5}$ | 25 | .01156 | .08832 |
| 6-15 | 4 | $22^{2}$ | $27^{3}$ | $25^{.5}$ | 19 | .01384 | .16736 |
| 7-f | 3 | $10^{0}$ | $10^{1}$ | $10^{.5}$ | 43 | .00703 | .08379 |
| 7-15 | 4 | $47^{0}$ | $94^{1}$ | $70^{2.5}$ | 7 | .02218 | .17570 |
| 8-f | 4 | $34^{2}$ | $82^{3}$ | $48^{2.5}$ | 13 | .01699 | .17051 |
| 8-15 | 3 | $22^{0}$ | $14^{3}$ | $18^{1.5}$ | 23 | .01227 | .08903 |
| 9-f | 3 | $19^{2}$ | $24^{3}$ | $22^{.5}$ | 20 | .01342 | .09018 |
| 9-15 | 3 | $76^{2}$ | $47^{3}$ | $62^{.5}$ | 10 | .01920 | .09596 |
| 10-f | 4 | $13^{0}$ | $6^{3}$ | $9^{3.5}$ | 45 | .00664 | .16016 |
| 10-15 | 5 | $5^{2}$ | $5^{1}$ | $5^{1.5}$ | 93 | .00056 | .31261 |
| 11-f | 5 | $7^{1}$ | $8^{1}$ | $7^{3}$ | 58 | .00453 | .31157 |
| 11-15 | 5 | $11^{1}$ | $12^{1}$ | $11^{3}$ | 38 | .00806 | .31510 |
| 12-f | 4 | $71^{1}$ | $45^{3}$ | $58^{3}$ | 11 | .01841 | .17193 |
| 12-15 | 3 | $9^{0}$ | $6^{2}$ | $7^{3}$ | 58 | .00453 | .08129 |
| 13-f | 3 | $11^{0}$ | $11^{1}$ | $11^{.5}$ | 40 | .00764 | .08440 |
| 13-15 | 4 | $12^{1}$ | $11^{1}$ | $11^{3}$ | 38 | .00806 | .16158 |
| 14-f | 4 | $15^{2}$ | $96^{2}$ | $56^{0}$ | 11 | .01841 | .17193 |
| 14-15 | 3 | $5^{0}$ | $6^{1}$ | $5^{2.5}$ | 88 | .00109 | .07785 |
| 15-f | 4 | $22^{3}$ | $20^{2}$ | $21^{2.5}$ | 20 | .01342 | .16694 |
| 15-15 | 4 | $39^{3}$ | $13^{1}$ | $26^{2}$ | 19 | .01384 | .16736 |
| 16-f | 4 | $32^{3}$ | $77^{2}$ | $55^{.5}$ | 12 | .01766 | .17118 |
| 16-15 | 5 | ** | | | | | |

## TABLE III (cont.)

| | | | | | | | |
|---|---|---|---|---|---|---|---|
| 17-f | 4 | $22^1$ | $18^3$ | $20^2$ | 21 | .01302 | .16654 |
| 17-15 | 4 | $81^2$ | $49^3$ | $65^{2.5}$ | 9 | .02006 | .17358 |
| 18-f | 4 | $9^3$ | $10^1$ | $10^0$ | 44 | .00684 | .16036 |
| 18-15 | 4 | $8^2$ | $8^3$ | $8^{2.5}$ | 51 | .00561 | .15913 |
| 19-f | 3 | $54^2$ | $43^0$ | $48^3$ | 13 | .01699 | .09375 |
| 19-15 | 4 | $9^2$ | $10^2$ | $10^0$ | 44 | .00684 | .16036 |
| 20-f | 3 | $30^3$ | $30^0$ | $30^{1.5}$ | 18 | .01428 | .09104 |
| 20-15 | 4 | $6^1$ | $6^0$ | $6^{.5}$ | 77 | .00219 | .15571 |
| 21-f | 2 | $7^1$ | $6^0$ | $6^{2.5}$ | 72 | .00275 | .04113 |
| 21-15 | 4 | $6^2$ | $6^2$ | $6^2$ | 73 | .00263 | .15615 |
| 22-f | 3 | $81^2$ | $66^3$ | $74^{.5}$ | 7 | .02218 | .09894 |
| 22-15 | 2 | $7^3$ | $5^1$ | $6^2$ | 73 | .00263 | .04101 |
| 23-f | 4 | $9^3$ | $10^0$ | $9^{3.5}$ | 44 | .00684 | .16036 |
| 23-15 | 4 | $10^1$ | $12^0$ | $11^{.5}$ | 40 | .00764 | .16116 |
| 24-f | 5 | $12^2$ | $13^0$ | $12^3$ | 35 | .00876 | .31580 |
| 24-15 | 4 | $15^0$ | $15^3$ | $15^{1.5}$ | 28 | .01062 | .16414 |
| 25-f | 4 | $47^0$ | $94^2$ | $73^0$ | 7 | .02218 | .17570 |
| 25-30 | 4 | $42^3$ | $43^2$ | $43^{.5}$ | 14 | .01640 | .16992 |
| 26-f | 5 | $6^0$ | $5^3$ | $5^{3.5}$ | 83 | .00152 | .30856 |
| 26-15 | 4 | $6^2$ | $6^1$ | $6^{1.5}$ | 75 | .00238 | .15590 |
| 27-f | 2 | $40^1$ | $38^0$ | $39^{.5}$ | 16 | .01526 | .05364 |
| 27-15 | ** | | | | | | |
| 28-f | 1 | $12^2$ | $9^2$ | $11^0$ | 40 | .00764 | .02683 |
| 28-15 | 3 | $10^2$ | $12^2$ | $11^2$ | 39 | .00756 | .08450 |
| 29-f | 0 | | | | | | |
| 29-10- | 3 | $27^2$ | $35^1$ | $31^{1.5}$ | 17 | .01476 | .09152 |
| 29-20 | 3 | $41^3$ | $40^1$ | $41^0$ | 15 | .01582 | .09258 |
| 30-f | 3 | $67^2$ | $83^1$ | $75^{1.5}$ | 6 | .02348 | .10024 |
| 30-15 | 3 | $20^1$ | $17^2$ | $18^{3.5}$ | 22 | .01261 | .08937 |
| 31-f | 3 | $10^3$ | $12^0$ | $11^{1.5}$ | 39 | .00783 | .08459 |
| 31-15 | 3 | $25^1$ | $23^1$ | $24^1$ | 19 | .01384 | .09060 |
| 32-f | 3 | $31^0$ | $52^3$ | $41^{3.5}$ | 15 | .01582 | .09258 |
| 32-15 | 2 | $24^1$ | $19^3$ | $22^0$ | 20 | .01342 | .05180 |
| 33-f | 1 | $7^1$ | $6^0$ | $6^{2.5}$ | 72 | .00275 | .02194 |
| 33-15 | 4 | $7^1$ | $7^0$ | $7^{.5}$ | 65 | .00361 | .15713 |
| 34-f | 4 | $25^0$ | $94^0$ | $59^2$ | 10 | .01920 | .17272 |
| 34-10 | 5 | ** | | | | | |
| 34-20 | 4 | $80^3$ | $95^3$ | $88^1$ | 3 | .02922 | .18274 |

| | | | | | | |
|---|---|---|---|---|---|---|
| 35-f | 0 | | | | | |
| 35-10 | 3 | $32^{1}$ | $52^{3}$ | $42^{2}$ | 15 | .01582 | .09258 |
| 35-20 | 3 | $32^{3}$ | $94^{1}$ | $63^{2}$ | 10 | .01920 | .09596 |
| 36-f | 4 | $17^{0}$ | $15^{1}$ | $16^{.5}$ | 27 | .01091 | .16443 |
| 36-15 | *** | | | | | | |
| 37-f | 3 | $17^{0}$ | $20^{1}$ | $18^{2.5}$ | 23 | .01227 | .08903 |
| 37-10 | 2 | $47^{1}$ | $9^{1}$ | $28^{1}$ | 18 | .01428 | .05266 |
| 37-20 | 3 | $8^{1}$ | $6^{2}$ | $7^{1.5}$ | 62 | .00397 | .08073 |
| 38-f | 4 | $10^{1}$ | $13^{1}$ | $11^{3}$ | 38 | .00806 | .16158 |
| 38-10 | 4 | $7^{1}$ | $7^{2}$ | $7^{1.5}$ | 62 | .00397 | .15749 |
| 38-20 | 3 | $23^{1}$ | $12^{3}$ | $18^{0}$ | 24 | .01188 | .08864 |
| 39-f | 2 | $10^{3}$ | $10^{3}$ | $10^{3}$ | 41 | .00743 | .04581 |
| 39-10 | 2 | $14^{0}$ | $12^{2}$ | $13^{1}$ | 34 | .00899 | .04737 |
| 39-20 | 2 | $14^{1}$ | $12^{1}$ | $13^{1}$ | 34 | .00899 | .04737 |
| 40-f | 4 | $56^{0}$ | $37^{1}$ | $46^{2.5}$ | 14 | .01640 | .16992 |
| 40-15 | 4 | $9^{0}$ | $10^{3}$ | $9^{3.5}$ | 44 | .00684 | .16036 |
| 40-25 | 4 | $20^{2}$ | $32^{2}$ | $26^{2}$ | 19 | .01384 | .16736 |
| 41-f | 4 | $41^{0}$ | $53^{3}$ | $47^{1.5}$ | 13 | .01701 | .17053 |
| 41-15 | 4 | $10^{1}$ | $10^{3}$ | $10^{2}$ | 42 | .00724 | .16076 |
| 41-25 | 4 | $17^{1}$ | $13^{3}$ | $15^{2}$ | 28 | .01062 | .16414 |
| 42-f | 0 | | | | | | |
| 42-5 | 3 | $6^{2}$ | $6^{0}$ | $6^{1}$ | 77 | .00219 | .07895 |
| 42-10 | 3 | $32^{0}$ | $30^{1}$ | $31^{.5}$ | 17 | .01476 | .09152 |
| 42-20 | 3 | $8^{3}$ | $39^{0}$ | $23^{.5}$ | 19 | .01384 | .09060 |
| 43-f | 3 | $7^{1}$ | $6^{2}$ | $6^{3.5}$ | 68 | .00321 | .07997 |
| 43-10 | 4 | $51^{2}$ | $16^{0}$ | $33^{3}$ | 17 | .01476 | .16828 |
| 43-20 | 3 | $35^{0}$ | $11^{3}$ | $23^{1.5}$ | 19 | .01384 | .09060 |
| 44-f | 4 | $7^{2}$ | $9^{3}$ | $8^{2.5}$ | 51 | .00561 | .15913 |
| 44-5 | 4 | $9^{1}$ | $8^{0}$ | $8^{2.5}$ | 51 | .00561 | .15913 |
| 44-10 | 4 | $9^{0}$ | $6^{0}$ | $7^{2}$ | 60 | .00428 | .15780 |
| 44-20 | 4 | $8^{2}$ | $8^{3}$ | $8^{2.5}$ | 51 | .00561 | .15913 |
| 45-f | 2 | $11^{0}$ | $8^{2}$ | $9^{3}$ | 45 | .00664 | .04502 |
| 45-10 | 2 | $10^{1}$ | $12^{2}$ | $11^{1.5}$ | 39 | .00783 | .04621 |
| 45-20 | 2 | $14^{2}$ | $34^{2}$ | $24^{2}$ | 19 | .01384 | .05222 |
| 46-f | 2 | $7^{0}$ | $7^{2}$ | $7^{1}$ | 63 | .00386 | .04224 |
| 46-5 | 2 | $8^{0}$ | $12^{0}$ | $10^{0}$ | 44 | .00684 | .04522 |
| 46-10 | 2 | $7^{0}$ | $6^{0}$ | $6^{2}$ | 73 | .00263 | .04101 |
| 46-20 | 2 | $9^{0}$ | $7^{0}$ | $8^{0}$ | 56 | .00486 | .04324 |
| 47-f | 2 | $5^{1}$ | $5^{2}$ | $5^{1.5}$ | 93 | .00063 | .03901 |
| 47-5 | 2 | $5^{3}$ | $5^{3}$ | $5^{3}$ | 85 | .00138 | .03976 |
| 47-10 | 2 | $5^{2}$ | $5^{3}$ | $5^{2.5}$ | 88 | .00109 | .03947 |
| 47-20 | 2 | $8^{0}$ | $6^{2}$ | $7^{1}$ | 63 | .00386 | .08171 |

| | | | | | | | |
|---|---|---|---|---|---|---|---|
| 48-f | 2 | $6^0$ | $5^3$ | $5^{3.5}$ | 83 | .00152 | .03990 |
| 48-5 | 2 | $5^3$ | $5^3$ | $5^3$ | 85 | .00138 | .03976 |
| 48-10 | 2 | $8^2$ | $8^2$ | $8^2$ | 52 | .00543 | .04381 |
| 48-20 | 2 | $15^0$ | $9^1$ | $12^{.5}$ | 37 | .00828 | .04666 |
| 49-f | 3 | $9^1$ | $9^0$ | $9^{.5}$ | 48 | .00611 | .08287 |
| 49-15 | 3 | $45^0$ | $52^2$ | $48^3$ | 13 | .01701 | .09377 |
| 50-f | 5 | * | | | | | |
| 50-10 | 5 | * | | | | | |
| 50-20 | 5 | * | | | | | |

*By the end of two hours these specimens showed complete digestion through the fifth tube but a sixth could not be done because too little of the specimen remained. Experience had indicated that digestion in the sixth would have been partial because of the slow rate of digestion in the fifth.

**Specimen not obtained because subject could not keep tube down.

***Specimen acid and could not be used.

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# THE LIPOLYTIC ACTIVITY OF THE DUODENAL CONTENTS

## Abstract of a Dissertation

Submitted in partial fulfilment of the requirements for the degree of
Doctor of Philosophy
BOSTON UNIVERSITY GRADUATE SCHOOL

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A clinically satisfactory method of lipolytic analysis for the duodenal contents must fulfill eight criteria: (1) and (2) the optimal pH and optimal temperature must be maintained during the course of the reaction; (3) inhibiting agents must be avoided and necessary activators or co-enzymes must be added; (4) operative variables in the system must be held to a minimum by the use of a fixed concentration of a suitable and evenly emulsified substrate; (5) the method of measuring the final extent of digestion must be accurate; (6) the enzyme must be protected from destructive influences in the interim between its procurement and its analysis; (7) the method must be simple, should preferably use ordinary laboratory apparatus and must consume as little time as possible; (8) the method must be designed to give as true a picture as possible of what might be expected to occur in vivo.

The following method is presented as more satisfactorily fulfilling these criteria than previously published methods; as substrate serves a 1% tributyrin emulsion rendered permanent by the addition of one gram of Alkanol B per one hundred cc. That is a DuPont product which they kindly stated is alkyl-naphthalene sodium sulfonate. This emulsion is stable after putting it twice through a hand homogenizer and will keep at least one month if kept in the ice box. The only other reagents required are three dilutions of sodium bicarbonate: 1.00%, 1.11%, and 1.25%. The method is one of serial dilution and all analyses are done in duplicate. Ten tubes are set up in pairs to contain the following reagents: tube (1) receives 4 cc. of 1.25% $NaHCO_3$; tube (2) 9 cc. of 1.11% $NaHCO_3$; tubes (3), (4), and (5) 5 cc. of 1.00% $NaHCO_3$. To tube (1) is added 1 cc. of the duodenal specimen to be analyzed and the same to tube (2). After thorough mixing 5 cc. of the contents of tube (2) are added to tube (3). In like manner 5 cc. of the contents of tube (3) are added to tube (4) etc. Five cc. are taken from the last tube of the series and discarded. In working with the duodenal contents a sixth tube is occasionally necessary and with experience one may predict that by noting the speed of digestion in the first tubes of the series immediately after the addition of the substrate. As soon as the various enzymic dilutions have been made, five cc. of the substrate are rapidly added to each tube. The tubes are then all well-stoppered, shaken and incubated in a water bath for two hours. The time interval begins the moment the substrate is added to the first tube and ends the moment the first tube is read in the colorimeter. The tubes should be read in the same order in which they are filled for within narrow limits it takes as long to fill them as it takes to read them in the colorimeter and in that way slight differences in the time of digestion for each tube are held to a minimum. Filter #565 is used for the colorimetric readings. Ninety-five per cent. of the light which it transmits lies in the range between 550 and 580 millimicra.

For comparative purposes single figures expressing the relative enzymic effects of each specimen were obtained in the following manner: it was assumed that each tube exhibiting complete digestion, as shown by the reading in the colorimeter and the grossly apparent clarification of the tube's contents, had present 1% of undigested substrate. By using the equation for calculating the velocity constant of a first order reaction and expressing the time in minutes, a value for the constant of 0.03838 was obtained. This figure was multiplied by the reciprocal of the dilution of the specimen in the last tube of the series showing complete digestion and to that product was added the constant calculated from the Evelyn readings of the first tube in the series to show incomplete digestion. For clinical purposes it suffices to note the number of tubes in the series revealing complete digestion.

The percentage of digestion of the substrate indicated by the colorimetric readings is determined by interpolation on a graph which represents the curve obtained by plotting the per cent. of undigested substrate against the colorimetric readings of standard dilutions of the substrate. For that purpose five tubes are prepared containing respectively 5, 4, 3, 2, and 1 cc. of the substrate diluted to a total volume, in each case, of 10 cc. with 1% $NaHCO_3$. In this case, as in all determinations, 10 cc. of 1% $NaHCO_3$ serves as a blank. The readings so obtained for different lots of emulsion do not vary by more than one quarter of a unit from the respective values: $5^0$, $6^0$, $7^2$, $11^0$ and $21^0$ where the superscripts as usual indicate quarter units.

The method just described was employed in the analysis of specimens obtained from fifty intubations. The subjects comprised ten hospital patients, twelve healthy nurses and twenty-one healthy medical students, of which latter group two were women. The activity in fasting specimens was found to range from zero to complete digestion through the fifth tube and partial digestion in the sixth. The specimens obtained at varying intervals, but usually fifteen minutes, after stimulation of the duodenal mucosa with twenty cc. of 0.1 N $HCl$ displayed activity varying from complete digestion through the second tube with partial digestion in the third to complete digestion through the fifth with partial digestion in the sixth. The latter group showed a sufficiently narrow range of dispersion to permit statistical analysis on the basis of the number of tubes showing complete digestion. The 64 specimens analyzed after stimulation gave a mean of 3.34 tubes with a standard deviation of 0.82 and a probable error of the mean of 0.07. The chance of finding normal cases showing complete digestion only through the first tube was found to be 22 in 10,000. The analogous figure for complete digestion through the sixth tube was 6 in 10,000. The same figures for the group exclusive of the ten hospital patients (all of whom, however, gave results falling within normal limits but tending to fall in the lower range of normal) were: mean, 3.80; standard deviation, 0.72; and probable error of the mean, 0.07. On the basis of this limited group the chance of finding a normal case with complete digestion only through the first tube was found to be one in 10,000 and the chance of finding a normal case revealing complete digestion through the sixth tube was found to be eleven in 10,000.

In fifteen cases at least two specimens obtained after stimulation were analyzed and in five of those, three specimens were analyzed. They usually showed complete digestion through the same number of tubes but in a few of the cases differed by one tube in that respect. It was concluded, therefore, that for clinical purposes the analysis of more than one specimen obtained after stimulation was not necessary.

Seven subjects were twice intubated. Of those seven, three gave specimens after stimulation of the same strength on the two different occasions. Of the remaining four two gave results differing by only one tube; one differed by two tubes on the two occasions; the results in the seventh case cannot be compared as a neutral or faintly alkaline specimen after stimulation was not obtained after one of the two intubations. With the subject whose specimens differed by two tubes the first intubation, which gave the lowest value, took an exceptionally long time. The results indicate a tendency for specimens obtained from the same individual on different occasions to be closely parallel in enzymic activity.

Reports in the literature indicate that such a test may have its greatest diagnostic value in cases of pancreatitis, steatorrhea, pancreatic and vicinal malignancies, pancreatic calculi and cysts and occasionally in biliary and hepatic diseases in which a secondary pancreatitis may be caused by the primary condition. The test may have prognostic value in following the course of a chronic pancreatitis.

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# AUTOBIOGRAPHY

I was born in Geneva, New York, August 21, 1910, the son of the Reverend David Howard Craver, D. D. and Mrs. Eva North Craver. I was educated in the public schools of Geneva until we moved to Auburn, New York, which has since been the family home. My public school education was completedupon graduation from Auburn Academic High School in 1928. That fall I matriculated at Cornell University, Ithaca, New York from which I was graduated in 1932 with the degree of A. B. in economics. From 1932 to 1934 I was instructor in Latin and Greek in the American College in Sofia, Bulgaria. It was there I met Elena Borikova and we were married August 17, 1933. Upon my return to this country in 1934 I began the study of chemistry at Boston University and was awarded my M. A. degree in the field of organic chemistry in 1936. In 1937 I became Teaching Fellow in Biochemistry at Boston University Medical School, a position I have held for four years. During that time I completed the first two years of medicine and my work in the field of biochemistry which latter lead to this attainment of the degree of Ph. D. in the field of biochemistry. During the past five years I have held various other part-time instructorships in chemistry at Boston University. At present we have one son, David Francis, who was born July 6, 1939. He has not yet settled on his field of study.

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